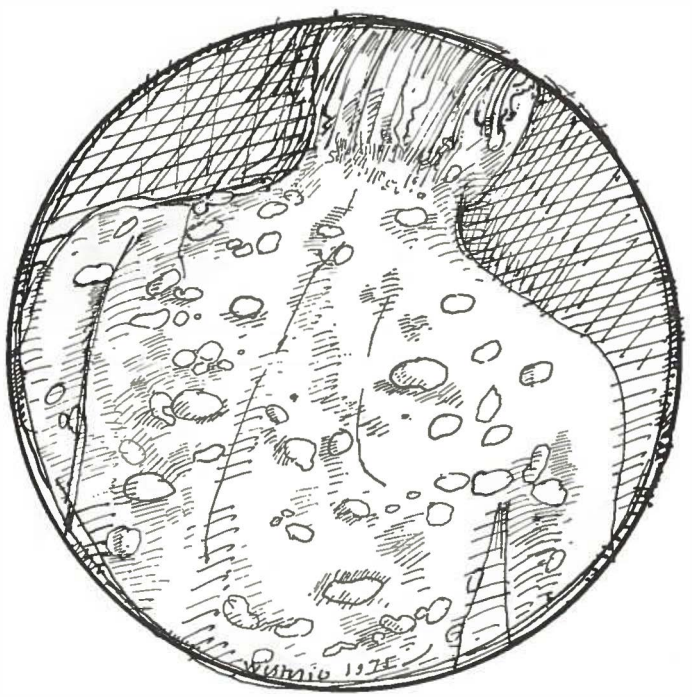
CHRONIC URTICARIA

H. M. G. DOEGLAS



CHRONIC URTICARIA

STELLINGEN

I

Het ziektebeeld vertraagde druk urticaria is onvoldoende bekend bij artsen en leidt daardoor niet zelden tot conflicten over de arbeidsgeschiktheid van deze patiënten.

II

Urticariële en asthmatische verschijnselen na aspirinegebruik, worden in het algemeen niet veroorzaakt door immunologische reacties.

III

De naam familiaire koude urticaria dient vervangen te worden door familiair koude exantheem.

IV

Kleine hoeveelheden nikkel in de voeding zijn mogelijk van belang voor het onderhouden van handeczeem van het dyshidrosiforme type bij nikkel overgevoelige patiënten.

O. B. Christensen et al. Contact Dermatitis 1, 136–141, 1975.

V

Atopie speelt geen rol van betekenis bij het ontstaan van penicilline allergie.

G. R. Green et al. J Allergy Clin Immunol 48, 331–343, 1971.
Ch. W. Parker, New Engl J Med 292, 511–514, 1975.

VI

De vorm van het recept dient uitsluitend bepaald te worden door de eis van optimale communicatie tussen arts, apotheker en patiënt.

VII

Het verdient overweging vitamine E therapie toe te passen bij afwijkingen waarbij een te snelle „turn over" van cellulaire membranen wordt vermoed.

VIII

Het is medisch gesproken onverdedigbaar, dat de algemene vaccinatie van Nederlandse zuigelingen tegen mazelen, twee en een half jaar na het uitbrengen van het advies ter zake door de Gezondheidsraad, nog niet wordt uitgevoerd.

IX

De prophylactische ontstolling met subcutaan toegediend calcium heparine heeft niet alleen effect op de frequentie van postoperatieve thrombose, maar ook op de frequentie van fatale longembolie.

Lancet 2, 45–51, 1975.

X

Het nuttig effect van het dragen van gymnastiekschoenen ter voorkoming van voetwratten, is te verwaarlozen.

XI

De zelfstandig gevestigde arts dient als bedreigde species beschermd te worden.

STELLINGEN BEHORENDE BIJ HET PROEFSCHRIFT VAN

H. M. G. DOEGLAS

CHRONIC URTICARIA

GRONINGEN 1975

RIJKSUNIVERSITEIT TE GRONINGEN

CHRONIC URTICARIA

CLINICAL AND PATHOGENETIC STUDIES IN 141 PATIENTS

PROEFSCHRIFT

TER VERKRIJGING VAN HET DOCTORAAT IN DE GENEESKUNDE
AAN DE RIJKSUNIVERSITEIT TE GRONINGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS DR. M. J. JANSSEN
IN HET OPENBAAR TE VERDEDIGEN
OP WOENSDAG 17 SEPTEMBER 1975 DES NAMIDDAGS TE 4 UUR

DOOR

HENDRIK MAARTEN GEORGE DOEGLAS
GEBOREN TE AMSTERDAM

1975
DRUKKERIJ DIJKSTRA NIEMEYER bv - GRONINGEN

PROMOTOR: PROF. DR. A. H. KLOKKE

COREFERENTEN: DR. E. BLEUMINK
DR. E. YOUNG

Aan mijn vrouw Audrey

en kinderen Alexandra, Mark en Hein

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The typing work was performed by mrs. J. A. C. de Ranitz and mrs. M. H. Brummer-Scholten. Miss R. H. J. M. Langermans, librarian of the Dermatology department aided in the literature search. The figures were drawn by mr. J. Fokkens.

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CONTENTS

INTRODUCTION

Chronic urticaria is a very troublesome disease due to its long duration, its frequent recurrences, the severe subjective symptoms and the impossibility to discover the etiology in many cases.

Urticaria is defined as an eruption of more or less transient erythematous or edematous swellings of the dermis or subcutaneous tissues. Urticaria and angioneurotic edema are often associated.[1]

A division in acute and chronic urticaria is made by most physicians and this division is based exclusively on the extension in time. Intervals of four weeks to six months have been used to define chronic urticaria We limited our study arbitrarily to patients with continuous or recurrent urticaria of more than three months duration.

We have considered the presence of physical urticarias an important element in the subdivision of our material.

Angioneurotic edema occurs in a large percentage of patients with urticaria, as an accompanying phenomenon, but it may also occur as the main symptom. It is considered a variant of urticaria without differences in etiology.

The following division may be made:

1. *physical urticarias*
   urticaria factitia
   delayed pressure urticaria
   cold urticaria (familial or acquired)
   cholinergic urticaria
   heat contact urticaria
   light urticaria
2. *urticaria with immunological causes*
3. *idiopathic urticaria*
4. *angioneurotic edema.*

PATHOGENESIS

Several reviews of the literature on the pathogenesis of urticaria have appeared recently.[2,3,4] A brief outline will be given here.

The histopathology of urticaria shows minimal changes consisting of edema, engorgement of small cutaneous blood vessels, dilated lymphatics and sometimes a slight perivascular infiltrate. The upper dermis is involved most in urticaria, the subcutaneous tissue in angioneurotic edema and delayed pressure urticaria. Electron microscopy shows exsudation of fluid through gaps between endothelial cells of the small venules.

Physiologically urticaria can best be described on the basis of Lewis' triple response consisting of erythema, edema and axon reflex flare. Lewis showed that the triple response could be elicited by a wide array of physical and chemical stimuli. The capacity of intracutaneous injection of histamine to mimic this reaction has focused the attention on histamine as a mediator of urticaria in general. Histamine is stored mainly in the granules of circulating basophilic granulocytes and cutaneous mast cells. In several forms of urticaria the lesions are caused by release of such mast cell depots. The knowledge that histamine plays a part in immediate type (atopic) hypersensitivity reactions has caused the widespread misconception that all urticaria must be allergic. Many recent publications however have studied the role of other mediator systems in urticaria such as the kallikrein-kinin system, the complement system and the prostaglandins.

The following pathogenetic factors must be considered:

1. *immunological factors.* The most important of these is the immediate type hypersensitivity reaction (type I of Gell and Coombs)[5], which is the typical reaction of atopy. Allergen is bound to IgE antibodies attached to the surface of mast cells or basophils, causing discharge of histamine and slow reacting substance of anaphylaxis (SRS-A). The role of SRS-A in the genesis of urticaria is not clear.

Less frequently urticaria accompanies cytotoxic reactions (type II of Gell and Coombs)[5], in which complement fixing antibodies attach to blood cells, such as in transfusion reactions or some types of drug rash. Finally urticarial lesions can develop during immune complex reactions (type III)[5] in which soluble immune complexes activate complement, after which anaphylatoxins are released. This type of reaction may occur in serum sickness, penicillin reactions, systemic lupus erythematosus and cryoglobulinemia. As Gell and Coombs[5] have pointed out combinations of these mechanisms may be present in one disease process.

2. *complement system.* The complement system consists of 9 main factors that can be activated successively, during which a number of media-

tors are formed. Some are able to cause discharge of mast cell granules such as the anaphylatoxins (C3a, C5a). Activation of complement is possible by immunological reactions (type II, III)[5], but also enzymatically Undue activation of the complement system is prevented by the presence of inhibitors in plasma. Congenital or acquired deficiencies in inhibitor levels will lead to an overflow of active mediators. A classical example is hereditary angioneurotic edema, in which the absence or inactivity of an inhibitor of the first component of complement, the C1 esterase inhibitor causes unchecked activation of complement, with formation of a vasoactive peptide which causes edema.

Secondly an alternate pathway of complement activation has been described, which bypasses the first components of complement and attacks C3. It is likely that certain urticarial reactions to high molecular compounds such as dextran act by this mechanism. Other possibilities are being investigated.

3. *chemical histamine liberators.* Some drugs and foods are able to cause discharge of mast cell granules by chemical means. This has been described with morphine, codeine, thiamine, polymyxine, d-tubocurarine and bee venom. Certain foods such as strawberries and lobster might cause recurrent urticaria in this way.

4. *the kallikrein-kinin system.* Circumstantial evidence has been produced, that the kallikrein-kinin system may be involved in urticarial reactions. Activation of the kallikrein system leads to the release of kinins (bradykinin, kallidin), which are potent vasoactive substances. Increased skin reactivity to kallikrein in urticaria has been described and there are conflicting reports about the presence of kinin activity in perfusates of urticarial reactions. Some authors reported decreased levels of protease inhibitors in cold urticaria.

5. *the prostaglandins.* The prostaglandins are mediators that can be formed locally in the skin from fatty acid precursors by a group of enzymes referred to as prostaglandin synthetases. Prostaglandin E_1 has a potent and longlasting vasodilating effect accompanied by pain and not by itching, on intracutaneous injection into the skin. It may also potentiate the effect of histamine and kinins. The exact role of the prostaglandins in the genesis of urticaria is undetermined.

6. *physical factors.* Physical factors such as scratching, heavy pressure, local or generalized heat or cold and ultraviolet light may cause urticaria

in some patients. This group of urticaria patients is of great interest because the lesions can often be created at will and offer possibilities for investigation. However knowledge of the pathogenesis is fragmentary. Lewis first demonstrated the triple reponse in patients with urticaria factitia. In cold contact urticaria increased levels of histamine have been demonstrated in the blood. In cholinergic urticaria a nervous impulse along cholinergic fibers is required. Finally in urticaria factitia, cold urticaria and cholinergic urticaria, positive Prausnitz-Küstner reactions have been demonstrated in some patients. This is not a proof for the presence of immunological processes because mediators might be transferred directly in the experiment.

7. *genetic causes of urticaria.* In hereditary angioneurotic edema, the biochemical defect is known and consists of an absent or biologically inactive inhibitor of the C1 esterase. Possibly similar mechanisms play a role in the causation of familial cold urticaria, familial vibratory angioedema or familial localized heat contact urticaria.

Of course it is possible that multiple causative factors may be at work in one particular type of urticaria.

GENERAL OUTLINE OF INVESTIGATIONS

In the present study the following subjects were investigated:

1. immunological aspects of the pathogenesis of chronic urticaria.
2. the role of aspirin sensitivity and sensitivity to food additives in chronic urticaria.
3. the role of protease inhibitors in the pathogenesis of chronic urticaria.
4. the effect of cold exposition on protease inhibitor values in patients with familial cold urticaria.
5. genetic linkage between the familial cold urticaria locus and marker genes.

The results of these investigations are reported in five successive articles, forming Chapters 1 to 5 of this thesis.

1. *immunological aspects of chronic urticaria.* In the past various intercurrent diseases have been designated as 'cause' for the urticaria. The introduction of control groups demonstrated, that such findings as a rule were not statistically significant. Immediate type hypersensitivity reactions (type I) are especially found in atopic patients showing reactions with foods, drugs and inhalants. Immune complex reactions (type III) are

possible in a great variety of conditions, such as drug reactions (penicillin), cryoglobulinemias, some infectious diseases and systemic lupus erythematosus.

We compared the incidence of atopy in patients with chronic urticaria with that in a control group of patients with other skin diseases. Drug reactions, especially to penicillin, were studied by history and skin tests.

The role of infections with bacteria, viruses, fungi and parasites was studied by clinical and laboratory methods and the effect of treatment of the infections was evaluated.

Intercurrent diseases known to be associated with immunological phenomena were diagnosed and evaluated for a possible connection with the urticaria.

2. *aspirin sensitivity in chronic urticaria patients.* It is known that aspirin sensitivity occurs in a high percentage of patients with chronic urticaria. The study of the role of aspirin sensitivity received a new impulse by the recent finding that aspirin sensitive urticaria patients may also show urticarial reactions on ingestion of some chemically non related food and drug additives. Certain azo dyes, especially tartrazine which is used extensively in foods and drugs and benzoates used as preservatives in foods and also in drugs, have given reactions in provocation tests in a high percentage of aspirin sensitive urticaria patients. The possibility that chronic urticaria is maintained by repeated exposure to certain food additives offered new therapeutic possibilities.

Because the history of aspirin reactions alone appeared unreliable, oral provocation tests with aspirin were performed in all urticaria patients. In patients that could be shown to be sensitive to aspirin, a range of food additives and other analgesics was tested as well. A diet free of additives, based on the laws regulating the use of additives in the Netherlands was given to all patients with positive provocation tests.

3. *plasma protease inhibitors in chronic urticaria.* It has been suggested recently that a deficiency in certain plasma protease inhibitors might play a role in the pathogenesis of chronic urticaria. A link between edema formation and protease inhibitors has been established, when it was found that patients with hereditary angioneurotic edema have a deficiency in C1 esterase inhibitor. Some authors noticed increased skin reactivity in patients with chronic urticaria to intracutaneous injection with kallikrein, a protease that splits vasoactive peptides from serum globulin. They postulated a lack of kallikrein inhibitor in patients with chronic urticaria.

Others observed deficiencies in protease inhibitors in patients with cold urticaria.

Plasma protease inhibitors such as C1 esterase inhibitor, α_1-antitrypsin, α_2-macroglobulin and the inhibiting capacities of plasma for trypsin, chymotrypsin and kallikrein are responsible for the equilibrium of several mediator systems. These include the complement, the kallikrein-kinin system and the fibrinolysis system. Absence or inactivity of inhibitors might cause an overflow of mediators such as bradykinin or anaphylatoxin, that are able to induce vasopermeability or histamine release respectively.

Therefore levels of these inhibitors in plasma were measured in the various groups of chronic urticaria patients and in controls.

4. *familial cold urticaria, clinical findings and plasma protease inhibitor values after cold exposure.* Two patients with this rare autosomal dominant hereditary disease were present in the group of patients collected in the study period. Because of the known hereditary nature of the disease, this family was described separately from the other 141 patients. The entire family was examined and 11 patients were found among 19 subjects in three generations.

The symptoms could be reproduced, by exposition to a cold room of 4 °C, in two patients. The changes in plasma protease inhibitor values were studied in these two patients, before, during and after exposure.

5. *familial cold urticaria, genetic linkage studies.* All 19 members of the family were investigated for the presence of 31 genetic markers, with the purpose of performing linkage analysis between each of the loci or chromosomal regions for these markers and the cold urticaria locus. The genetic markers included blood groups, serum groups, red cell enzymes and polymorphic chromosomes.

REFERENCES

1. Champion RH: Urticaria, in Rook A, Wilkinson DS, Ebling FJG (eds): *Textbook of Dermatology*, ed 2, Oxford, Blackwell Scientific Publications, 1972, vol 1, pp 902–908.
2. Warin RP, Champion RH: *Urticaria. Major Problems in Dermatology*, vol 1, London, W. B. Saunders Company Ltd, 1974, pp 1–173.
3. Mathews K: A current view of urticaria, in Samter M (ed): Allergy in adults, *Medic Clinics North America* 58, 185–205, 1974.
4. Valentine MD, Sheffer AL, Austen KF: Urticaria and angioedema, in: Samter M (ed): *Immunological Diseases*, ed 2, Boston, Little Brown and Company, 1971, vol 2, pp 907–919.
5. Gell PH, Coombs RA: Clinical aspects of immunology, ed 2, Oxford, Blackwell Scientific Publications, 1968, pp 583–588.

CHAPTER 1

INCIDENCE, CLASSIFICATION AND IMMUNOLOGICAL ASPECTS OF CHRONIC URTICARIA

INTRODUCTION

Allergy has played a dominant role in the thinking on the etiology of urticaria, since the work of Wolf-Eisner (1907).[1] The ideas about its relative importance are far from settled however. Figures for allergic causes of urticaria range from 3 to more than 50 %[2,3] and the incidence of atopy in urticaria populations is estimated at 15 to 60 %.[2,4] When control series are included the incidences were usually similar.[2] Dermatologists have often stressed that in most urticaria patients an allergic origin cannot be demonstrated.[5,6] Nevertheless classifications giving a predominant place to immunological causes are still being published.[7] In any case it is known that type I allergic reactions (atopic) and to a lesser degree type III (immune complex disease) can be accompanied by urticarial lesions.[8]

We have compared the incidence of atopy in our urticaria patients with that in a control group of patients with other skin diseases, matched for sex and age. Atopy was defined on the basis of the history of the patient and his first degree relatives and the result of skin tests with a number of well defined atopic allergens.

Urticarial reactions to drugs can be immediate type reactions (type I), or can be part of serum sickness type reactions (type III). It has been shown that penicillin reactions constitute the majority of drug reactions found in urticaria patients.[9-11] Penicillin is also the only drug for which reliable skin tests are available. For these reasons, the role of penicillin allergy was studied in our patients by history, skin tests and a diet free of traces of penicillin.

The role of infections with bacteria, viruses, fungi and parasites in the etiology of chronic urticaria is poorly defined. Type I reactions to microbial antigens and type III reactions to antigen-antibody complexes have to be considered.

The role of microbial antigens in chronic urticaria cannot be proven by

skin tests, serological reactions or immunofluorescent techniques such as has been reported in patients with vasculitis.[12] However urticaria has been described as a symptom in infectious diseases such as virus and serum hepatitis,[13, 14] ornithosis,[15] melioidosis,[16] and tropical parasitic diseases such as filariasis,[17] strongyloidosis,[18] and malaria.[19]

We limited our study to the diagnosis and treatment of infections and infestations and evaluated the results of treatment on the urticaria. The incidence of intercurrent diseases was studied,[20] including those known to be associated with immunological phenomena such as systemic lupus erythematosus (SLE) and vitiligo.

PATIENTS, MATERIALS AND METHODS

Patients

All patients with urticaria with a duration of over three months, seen in the period July 1, 1971 to July 1, 1974 were included. Patients who had known drug hypersensitivities with the exception of aspirin sensitivity and patients with internal diseases known to cause urticaria and known to exist already at the beginning of the study were excluded. Also excluded were patients in whom urticaria had its onset in combination with vasculitis, purpura, erythema nodosum or erythema multiforme.

History

The history has proven to be one of the most effective means of investigation in urticaria.[2, 10] A questionnaire was designed with specific questions regarding known causes of urticaria. Physical urticarias often show a relationship in time or localization to trauma, temperature changes or certain activities. The patients were questioned about the use of foods, drugs, inhalants or contact substances which may cause type I or III allergic reactions, or cause histamine relase directly.

Specific questions were asked about focal infections of oral and nasal regions, worm infections and infectious diseases or internal diseases known to cause urticaria. The presence of mental problems, psychiatric treatment or hospital admissions was noted.

A careful family history was obtained in particular of the presence of atopic disorders and of urticaria and angio edema.

Physical examination

All patients were examined for the presence of skin lesions, lesions of the mucous membranes, hairs and nails. In particular the presence of fungus infections, vitiligo as well as urticarial lesions was noted.

Control group

For the determination of the incidence of atopy in urticaria patients, a control group was collected of patients with other skin diseases from the same clinic. Patients who were suffering from eczematous diseases or internal diseases were excluded. The control group was taken proportionally from the same sex and age groups as the patient group. The control group consisted of 53 patients, 32 women and 21 men compared to 141 patients in the urticaria group with 73 women and 68 men. The mean age in the control group was 33.2 years, the median 30.0 years. In the patient group these figures were respectively 33.1 years and 30.0 years.

The patients in the control group had psoriasis, acne vulgaris, crural ulcers or venereal disease.

Tests for physical urticaria

These are described in chapter 3, pages 47 and 48.

Intracutaneous tests

Intracutaneous tests were performed with the food allergens cow's milk (lactulose-ß lactoglobulin) and egg white.[21] The following inhalants were used: house dust (0.1 %), human dander (0.1 %), mixed animal dander (0.01 %), mixed fungi (0.1 %), hay (0.01 %), mixed grass pollen (100 Noon units/ml), mixed tree pollen, spring pollen (100 Nu./ml), and saline phosphate buffer pH 6.9 as a control. (Haarlem Allergen Lab.) The reactions were read after 20 minutes and compared with those to histamine solutions of 0.1, 0.01 and 0.001 mg histamine/ml, scored as 1+, 2+ and 3+ reactions respectively. Penicillin tests were done, first as scratch tests with penicilloyl polylysin (10^{-6} M), benzyl penicillin (10^{-2} M) and a saline phosphate buffer as control. If these tests were negative, intracutaneous tests were done with penicilloyl polylysin (10^{-6} M), benzyl penicillin (10^{-2} M), a minor determinant mixture of sodium penilloate (10^{-2} M) and sodium penicilloate (10^{-2} M) and a

buffer control. These tests were done on the volar surface of the lower arm with 0.05 ml of the solution. The results were read after 20 minutes and measured in mm weal diameter. No attempt was made to read the reactions after 5–24 hrs. Reactions were considered positive when the weal diameter was over 5 mm and bigger than the control.

Tuberculin tests were performed with 0.05 ml P.P.D. 1/10.000, the reaction was read after 48 hrs. Reactions less than 6 mm were considered negative.

Intracutaneous tests with commercial Candida albicans antigens were discontinued after it appeared that there was a wide divergence in results. Positive tests occurred at all intervals, ranging from 10 minutes to 48 hours, without any correlation with clinical disease, both in patients with chronic urticaria and in controls.

Laboratory tests

The following tests were done in all patients: hemoglobin, hematocrit, total and differential white cell count, total eosinophil count, platelet count, erythrocyte sedimentation rate, urinalysis, cardiolipin complement fixation test, V.D.R.L. test, antistreptolysin titer, Rose test, latex fixation test, antinuclear fluorescence test, serum protein electrophoresis, cryoglobulin determination, alkaline phosphatase, lactic acid dehydrogenase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic acid transaminase, serum creatinin, urea and determination of Australia antigen and antibodies in serum.

In all patients with cold urticaria, cold agglutinins and cold hemolysins were determined.

In individual patients thyroid function tests (T3 and T4) and thyroid antibodies were determined. In patients with symptoms suggestive of SLE, LE cell clot tests, antibodies against DNA and organ specific antibodies were determined. In patients with vitiligo, antibodies against gastric parietal cell, thyroid, adrenal cortex and skeletal muscle were also determined.

Feces examination

Fresh stool specimens of all patients were collected on three successive occasions. A direct smear was examined for the presence of ova, worms and parasites.

For the diagnosis of oxyuriasis a cellophane adhesive tape swab of the perianal skin was taken and examined on a glass slide covered with olive oil.

Feces of patients who had been resident in the tropics, was also examined by the parasitology laboratory of the Institute for Tropical Hygiene in Amsterdam with a zinc sulphate, centrifugation, flotation method (Faust [22]) and a formaldehyde, ether, sedimentation method (Ritchie [23]).

Examination of material for fungi

Skin scrapings or material collected from nails of patients clinically suspected of fungus infections, was examined microscopically after standing for 6 hours in a 20 % potassium hydroxide solution in water. Specimens were cultured on a medium consisting of Sabouraud dextrose agar (Difco) with actidion, chloromycetin, penicillin, streptomycin and vitamin B_1 and also on a medium of Littman oxgall agar (Difco).

Focal infections

Attention was concentrated on the oral and paranasal regions. Other infections were investigated if their presence was suggested by the history. All patients received a chest X-ray and were examined by the departments of Oral Surgery and Otorhinolaryngology, with the purpose of detecting dental infections and polyposis or sinusitis. Vitality tests of the teeth were done by hot and cold application or by electric stimulation. Dental roentgenograms were taken in all patients.

Roentgenograms of the paranasal sinuses were made when there was the slightest suspicion of abnormalities. Treatment was instituted when abnormalities were detected.

RESULTS

Prevalence and classification

The prevalence of chronic urticaria over the three year period of study was 1.1 % or 185 cases among 15.675 new patients in the outpatient department. Of these 141 completed the study. The reasons for not completing the study in 44 patients, were mostly lack of cooperation or time

TABLE 1. TYPES OF URTICARIA AMONG 141 PATIENTS WITH CHRONIC URTICARIA

	number of patients			percentage
	male	female	total	
physical urticarias				
urticaria factitia	8	17	25	18
pressure urticaria	11	6	17	12
cold urticaria	7	11	18	13
cholinergic urticaria	11	6	17	12
heat contact urticaria	1	—	1	—
total	38	40	78	55
idiopathic urticaria	26	30	56	40
angioneurotic edema	4	3	7	5
total	68	73	141	100

on the side of the patient. In the same period a diagnosis of acute urticaria was made in only 31 patients (0.3 %). Almost all patients were studied as outpatients.

The different types of urticaria diagnosed are shown in table 1. The results of the tests for physical urticaria afforded a natural basis for classification. In patients with more than one type of physical urticaria, the predominant symptomatology decided upon the final classification. There were 73 women and 68 men.

The average duration of the skin lesions, at the time the patients were first seen, was 2.3 years, with a range of three months to 27 years (Table 2). It can be seen that the duration of the physical urticarias, with the exception of cholinergic urticaria, exceeds that of idiopathic urticaria.

TABLE 2. AVERAGE DURATION* OF CHRONIC URTICARIA IN 141 PATIENTS

	male	female	total
urticaria factitia	2.6	2.4	2.6
pressure urticaria	3.3	0.7	2.4
cold urticaria	4.1	4.4	4.2
cholinergic urticaria	1.4	1.3	1.4
heat contact urticaria	1.0	—	1.0
idiopathic urticaria	1.5	2.0	1.8
angioneurotic edema	0.7	3.0	2.0
total	2.3	2.3	2.3

* duration in years, at first visit.

TABLE 3. AVERAGE AGE* AT ONSET IN 141 PATIENTS WITH CHRONIC URTICARIA

	age		
	male	female	total
urticaria factitia	28	22	24
pressure urticaria	37	38	37
cold urticaria	24	25	24
cholinergic urticaria	23	25	24
idiopathic urticaria	34	25	30
angioneurotic edema	30	16	21
total	33	23	27

* age given in years.

The average age at onset of the urticaria (Table 3) was 27 years, 33 for the men and 23 for the female patients. The age at onset in the various types of urticaria is more or less similar, ranging from 20 to 30 years. The patients with pressure urticaria seem to be older at onset with an average of 37 years.

Overlapping between the diagnostic groups did occur. Urticaria factitia was present in one patient each, in the groups with pressure urticaria, cold urticaria, cholinergic urticaria and idiopathic urticaria. Asymptomatic dermographism alone occurred in a further two patients with cold urticaria, one with cholinergic urticaria and three with idiopathic urticaria. Combinations with idiopathic urticaria were seen in 15 of 17 patients with pressure urticaria, 4 of 25 with urticaria factitia, 4 of 17 with cholinergic urticaria and one patient with angioneurotic edema.

Occasional angioneurotic edema, present at least once, was seen in 46 out of 56 patients with idiopathic urticaria, in 11 out of 17 with pressure urticaria, in 5 out of 17 with cholinergic urticaria and in 4 out of 25 with urticaria factitia. In patients with cold urticaria it is hard to evaluate spontaneous angioneurotic edema, because cold exposure often will cause marked swelling. Cold urticaria was rarely seen in combination with other types of urticaria. In the group with cholinergic urticaria, a history of reactions to cold, similar to those evoked by sweating, was present in 6 patients. Notwithstanding intensive testing this cold sensitivity could be verified in only one patient. This 26 year old female developed the same type of rash, typical for cholinergic heat urticaria, after exposure to a half full bath of cold water of 15°. Apparently she suffered both from cholinergic (reflex) heat urticaria and reflex cold urticaria.

Prevalence of atopy in chronic urticaria patients and controls

The diagnosis of atopy was based on the presence of one or more positive intracutaneous tests with house dust, human or animal dander in the patient, together with evidence of asthma, seasonal hay fever or atopic dermatitis in the patient or his first degree relatives (parents, siblings, children). The same data were collected of controls and their first degree relatives.

Using this definition, (Table 4) a higher percentage of atopics is found in the groups with idiopathic urticaria and urticaria factitia. These differences are significant ($p = 0.01$–0.02, with Student's t test). Similarly the largest number of positive tests with a variety of allergens is also found in the groups with idiopathic urticaria and urticaria factitia (Table 5).

If we compare the prevalence of a history of atopic diseases in the patients and their first degree relatives with that in a control group, there are no important differences (Table 6).

Patients with positive skin tests in general did not have a history of urticaria after exposure to the allergen in question. There was one patient with idiopathic urticaria with positive skin tests to pollen and seasonal exacerbations in the summer and one atopic patient with angioneurotic edema, positive to house dust and human dander, who had attacks of asthma and angio edema after exposition to dusty environments.

Dermographism has been suggested to be the cause of multiple false positive reactions to intracutaneous tests. In the 25 patients with urticaria factitia, factitious weals at all injection sites including the controls, were seen in only three patients. These three patients were not included among the six diagnosed as atopics.

TABLE 4. PREVALENCE OF ATOPY IN PATIENTS WITH CHRONIC URTICARIA AND CONTROLS

	number of patients	number of atopics	percentage	significance
urticaria factitia	25	6	25	$p = 0.02$
pressure urticaria	17	2	12	n.s.
cold urticaria	18	2	11	n.s.
cholinergic urticaria	17	2	12	n.s.
idiopathic urticaria	56	15	27	$p = 0.01$
angioneurotic edema	7	1	14	n.s.
controls	53	3	6	

n.s. = not significant

TABLE 5. FREQUENCY OF POSITIVE INTRACUTANEOUS TESTS IN CHRONIC URTICARIA AND CONTROLS

	N	house dust	human dander	hay	grass pollen	spring pollen	anim dander	fungi	egg, milk
urt factitia	25	6(24%)	11(44%)	4(16%)	2 (8%)	1 (4%)	6(24%)	1 (4%)	5(20%)
pressure urt	17	3(18%)	5(30%)	1 (6%)	1 (6%)	1 (6%)	1 (6%)	1 (6%)	2(12%)
cold urticaria	18	2(11%)	4(22%)	2(11%)	1 (6%)	2(11%)	1 (6%)	0 (0%)	1 (6%)
cholinergic urt	17	3(18%)	2(12%)	1 (6%)	2(12%)	1 (6%)	2(12%)	0 (0%)	2(12%)
idiopathic urt	56	19(34%)	18(32%)	9(16%)	11(20%)	12(21%)	9(16%)	6(11%)	11(20%)
angioneurotic ed	7	1(14%)	2(29%)	0 (0%)	1(14%)	1(14%)	0 (0%)	1(14%)	0 (0%)
total	140	37(26%)	51(36%)	18(13%)	19(14%)	22(16%)	21(15%)	11 (8%)	22(16%)
controls	53	3 (6%)	9(17%)	1 (2%)	1 (2%)	4 (8%)	2 (4%)	3 (6%)	1 (2%)

Another type of non specific reaction occurred 2 to 5 hours after intracutaneous tests, the drawing of blood or insect bites. This was reported particularly by patients with delayed pressure urticaria and also by some patients with idiopathic urticaria. No systematic inspection of injection sites was performed after 48 hours. The 17 patients with delayed pressure urticaria were questioned about such reactions and delayed reactions were reported four times after intracutaneous tests, eight times after the drawing of blood and three times after insect bites. This phenomenon deserves further study.

Drug allergy

Penicillin is one of the drugs most frequently causing urticarial rashes. Ten of our patients had, a history of reactions after the use of penicillin, positive skin tests to penicillin antigens, or both (Table 7). However in the six patients with a history of penicillin reactions, only three had positive skin tests (cases 5, 6, 10) and in these patients there was no relationship in time between the penicillin reaction and the onset of chronic urticaria.

In four patients (cases 1, 4, 7, 9) there was no history of penicillin reactions, but the skin tests with penicillin antigens were positive. Two patients with positive skin tests to penicillin antigens (cases 9, 10) were cured of their urticaria after the use of a diet free of milk and milk products in order to avoid foods possibly containing traces of penicillin. It is noteworthy that both patients who responded to a diet, were in the group of idiopathic urticaria and not among those with physical urticarias.

TABLE 6. PREVALENCE OF ATOPY IN THE HISTORY OF PATIENTS WITH CHRONIC URTICARIA AND CONTROLS

	number	own history	history in first degree relatives
urticaria factitia	25	2 (8%)	10 (40%)
pressure urticaria	17	3 (18%)	3 (18%)
cold urticaria	18	10 (56%)	8 (44%)
cholinergic urticaria	17	0 (0%)	6 (35%)
idiopathic urticaria	56	4 (7%)	29 (52%)
angioneurotic edema	7	1 (15%)	4 (57%)
total	140	11 (8%)	60 (43%)
controls	53	7 (13%)	17 (32%)

Notwithstanding exhaustive questioning, we have been unable to ascertain any other clear cut relationships between the use of drugs and the onset or exacerbation of urticaria, with the exception of aspirin and related chemicals. The role of aspirin sensitivity in chronic urticaria is considered separately in chapter 2.

TABLE 7. HISTORY OF PENICILLIN REACTIONS AND PREVALENCE OF POSITIVE SKIN TESTS WITH PENICILLIN ANTIGENS IN PATIENTS WITH CHRONIC URTICARIA AND CONTROLS

case	sex, age at onset	onset urtic	penic reaction	results of intracutaneous tests with P.P.L.	benzyl penic	minor determ	atopy	effect diet
urticaria factitia								
1.	M.18	1964	–	–	–	+	+	–
2.	F.18	4/73	5/73	–	–	–	+	–
pressure urticaria								
3.	M.49	9/72	9/72	–	–	–	–	–
4.	M.34	1956		+	–	–	–	–
5.	M.36	1962	1967	+	+	–	–	–
cold urticaria								
6.	F.25	1964	1967	+	–	–	–	–
cholinergic urticaria								
7.	M.27	1973	–	–	–	+	–	–
idiopathic urticaria								
8.	M.32	1964	1964	–	–	–	–	–
9.	F.25	1971	–	+	+	–	–	+ (4/72)
10.	F.29	1971	9/73	+	–	–	+	+ (9/73)
controls								
(53)		–	–	–	–	–	3	.

TABLE 8. PREVALENCE OF OTORHINOLARYNGOLOGICAL INFECTIONS AND RESULTS OF TREATMENT IN PATIENTS WITH CHRONIC URTICARIA

	patients with abnorm findings	patients treated	improved
urticaria factitia	0/ 25	0	0
pressure urticaria	1/ 17	1	0
cold urticaria	1/ 18	1	0
cholinergic urticaria	3/ 17	2	0
heat urticaria	0/ 1	0	0
idiopathic urticaria	7/ 56	7	0
angioneurotic edema	0/ 7	0	0
total	12/141	11	0

In two patients other drugs appeared to be involved. However, the adverse reactions were not due to the drugs themselves, but to tartrazine or benzoates present as additives, once in an oral contraceptive and twice in a trichomoniacide.

Focal infections

Focal infections of the teeth and paranasal sinuses were examined. The results of the otorhinolaryngological examinations are shown in table 8. Maxillary sinusitis was present in one patient with cold urticaria, three with cholinergic urticaria, one with pressure urticaria and two with idiopathic urticaria. A pansinusitis was found in two patients with idio-

TABLE 9. PREVALENCE OF PERIAPICAL GRANULOMAS AND RESULT OF TREATMENT IN PATIENTS WITH CHRONIC URTICARIA

	patients with granulomas	number of granulomas	patients treated	improved
urticaria factitia	2/ 25	3	1	0
pressure urticaria	0/ 17	0	0	0
cold urticaria	3/ 18	5	3	0
cholinergic urticaria	3/ 17	11	3	1
heat urticaria	0/ 1	0	0	0
idiopathic urticaria	10/ 56	19	10	2
angioneurotic edema	0/ 1	0	0	0
total	18/141	38	17	3

TABLE 10. PREVALENCE OF OTHER DENTAL ABNORMALITIES IN PATIENTS WITH CHRONIC URTICARIA

	patients with abnorm findings	retained roots	periodontal disease and gingival pocket formation	impacted M3's	other
urticaria factitia	7/ 25	1	1	5	
pressure urticaria	6/ 17	1	2	1	1 abscess, 1 non vital pulp
cold urticaria	9/ 18	2	2	3	1 many deeply decayed teeth, 1 non vital pulp
cholinergic urticaria	0/ 17	0	0	0	
heat urticaria	1/ 1	1	0	0	
idiopathic urticaria	15/ 56	4	3	3	5 deeply decayed teeth, 1 sialolithiasis
angioneurotic edema	4/ 7	0	1	1	3 deeply decayed teeth, 1 calcified submental lymph nodes
total	42/141	9	9	13	14

pathic urticaria. In all patients the sinuses were irrigated and antibiotics prescribed. A purulent rhinitis was found in two patients with idiopathic urticaria. A streptococcal pharyngitis with a raised antistreptolysin titer occurred in one patient with idiopathic urticaria. In none of the patients treatment of the infection resulted in a cure of the urticaria.

The findings of the oral surgery department are given in tables 9 and 10. Periapical granulomas were the most frequent abnormality, 38 granulomas were found and treated, in 17 patients. This was followed by a cure of the urticaria within four weeks in five patients with idiopathic urticaria, two with cholinergic urticaria and one with cold urticaria. The observation period varied from nine months to two years. However in one patient with idiopathic and one with cholinergic urticaria, the cure may have also been the result of a diet free of additives introduced at the same time. In one patient with idiopathic and one with cold urticaria, the disease was of short duration (three months) and in one patient with idiopathic urticaria, the skin lesions had been in remission for some time before treatment, suggesting spontaneous cure. This leaves three cases (one cholinergic and two idiopathic) who might have benefited from oral surgical treatment. The average duration of the urticaria in these three patients was 12 months. This is considerably shorter than the average of the total group which was 2.3 years.

Other dental abnormalities (Table 10) were found in 42 patients. The most frequent findings were impacted third molars in 13, retained root tips in 9, deeply decayed teeth in 9, and periodontal disease with gingival pocket formation in 9. None of these patients improved after treatment, which is in agreement with the view that these lesions do not constitute focal infections.

One patient with urticaria factitia had an infected appendix removed during the period of study, without effect on his urticaria. In three patients gallbladder roentgenograms were made because of abdominal complaints, with negative results. None of our patients had had a cholecystectomy. A recurring pyelonephritis in one female patient with idiopathic urticaria, coincided with exacerbations of her urticaria. There was no relation with the drugs used for the infection.

Positive tuberculin tests were found in 26 patients of whom 7 had received B.C.G. Of the other 19 (average age 42.3 yrs.), 5 showed old inactive pulmonary lesions on chest X-ray and one of them had a nephrectomy for tuberculosis. In none of these patients active tuberculosis could be demonstrated by sputum examinations, urine cultures and tomograms. No other infiltrative processes of the lung could be demonstrated and there were no cases of pulmonary emphysema.

In all patients the history was evaluated for evidence of hepatitis and Australia antigen and antibody studies in the serum were performed. No active cases of hepatitis were encountered. In only one of 19 patients with a history of hepatitis and in one patient without a history, the reactions were positive. There was no clinical or laboratory evidence of liver damage or of active hepatitis in these patients.

Fungus infections of skin and nails were found in five patients, three with tinea pedis or onychomycosis caused by Trichophyton rubrum, two with tinea inguinalis (T. rubrum, T. mentagrophytes). All were treated with local fungistatics and oral griseofulvin, without effect on the urticaria.

Worm infestations

Feces samples and sellotape preparations were examined for parasitic infestation (Table 11).

There were 26 patients with worm infestations, 20 with oxyuris, six with ascaris and two with trichiuris trichiura infections. The prevalence of worm infestation in the 16 patients who had been in tropical areas,

TABLE 11. PREVALENCE OF WORM INFESTATION, EOSINOPHILIA AND RESULTS OF TREATMENT IN PATIENTS WITH CHRONIC URTICARIA

	N	patients treated	eosinoph > 300/mm³	cure
urticaria factitia	25	3 oxyuris	1	0
pressure urticaria	17	3 oxyuris	0	0
cold urticaria	18	2 oxyuris 1 ascaris	0	0
cholinergic urticaria	17	3 oxyuris 3 ascaris	1	1 ascaris*
heat contact urtcaria	1	—	0	—
idiopathic urticaria	56	10 { 8 oxyuris 2 ascaris 2 trich. tri	3	3 oxyuris
angioneurotic edema	7	1 oxyuris	0	0
total	141	26	5	4

* simultaneous removal of dental granuloma.

was equal to that in the remaining 125 patients, and there were no differences in the type of worm infestation between the two groups.

Patients with oxyuriasis and their families were treated with pyrvinium pamoate, 50 mg/kg, by mouth, in one dose, which was repeated after two weeks. Recurrences were treated with pyrantel pamoate, 10 mg/kg, in one dose, repeated after two weeks. Patients with ascariasis received piperazine adipate, 3.0 g per day, for two days, by mouth. Feces and perianal sellotape preparations were examined three times monthly after treatment.

Blood eosinophilia (over 300/mm³) was noted in five of 19 patients with worm infestations, of whom one had ascariasis and four oxyuriasis. In all five the number of eosinophils returned to normal after treatment of the worm infestation. This was independent of the effect on the urticaria, since in none of these patients there was any change in the course of the urticaria.

After treatment of the worm infestation, the urticaria disappeared within four weeks in three patients with idiopathic urticaria and oxyuriasis and in one patient with cholinergic urticaria and ascariasis. The average duration of the urticaria in these four patients was $1^1/_2$ years, and the duration of the follow up period one to two years. However the patient with ascariasis had a focal infection of the teeth treated at the same time. Therefore the possibility that treatment of worm infestation resulted in cure of the urticaria, has only to be considered in three of 25 patients.

Diseases associated with immunological phenomena

Under this heading some diseases are grouped that are known to be associated with immunological phenomena (Table 12).

There was evidence of subacute systemic lupus erythematosus in two patients. In a five year old girl, urticaria was followed after one year by malaise, weight loss, joint pains and fever. The antinuclear fluorescence test was positive continuously over several years, suspect LE cells were found intermittently, the sedimentation rate was elevated, but there were no leukopenia, hypergammaglobulinemia, antibodies against DNA or organ specific antibodies and no immune globulins could be demonstrated with immunofluorescence techniques in the skin. After five years the clinical condition remains unchanged.

The other patient was a man of 50 years, who developed subsequent to a penicillin reaction, a combination of spontaneous urticaria, pressure urticaria, aspirin sensitivity, vasculitis, fever and joint pains with an elevated sedimentation rate, eosinophilia, positive antinuclear fluorescence tests and LE cells and negative immunofluorescence of the skin.

TABLE 12. PREVALENCE OF DISEASES ASSOCIATED WITH IMMUNOLOGICAL PHENOMENA IN PATIENTS WITH CHRONIC URTICARIA

	sex age	onset urticaria	onset other disease	immunol reactions against	family members involved
urticaria factitia	F 31	1973	1971 vitiligo	—	—
	F 23	1971	1972 Hashimoto	thyroid-cytoplasm	—
pressure urticaria	M 50	1971	1973 SLE	antinuclear-fluorescence	—
cold urticaria	F 25	1965	1958 vitiligo	—	8
idiopathic urticaria	M 51	1971	1955 vitiligo	gastric-parietal cell	3
	M 53	1970	1920 vitiligo	thyroid-cytoplasm	—
	F 40	1965	1969 vitiligo sarcoidosis	gastric-parietal cell PPD−, DNCB+	—
	F 27	1966 1971	1965 sarcoidosis	PPD−, DNCB+	—
	F 6	1970	1971 SLE	antinuclear-fluorescence	—

The symptoms went in remission after treatment with prednisone and chloroquin.

Hashimoto's thyroiditis was diagnosed in a 21-year-old woman who developed a tender hard goiter with mild hypothyroidism, one year after the onset of urticaria factitia. There were circulating antibodies against thyroid cytoplasm. There was no relationship detectable between the symptoms of the two diseases.

Five cases of vitiligo were found. The vitiligo was familial in two patients. In four, the vitiligo preceded the urticaria by periods of two to fifty years, in one the vitiligo followed the urticaria by five years. In three patients there were autoantibodies, in two against gastric parietal cells, in one against thyroid cytoplasm. There was no relationship in localization, remissions or exacerbations between the urticaria and the vitiligo.

Sarcoidosis, a disease characterized by diminished cell mediated immunity, was diagnosed in two patients with idiopathic urticaria. One patient, a woman of 27 years, had sarcoidosis confirmed by scalene node biopsy from 1965 to 1971, with hilar adenopathy, pulmonary fibrosis and a negative tuberculin test. She had two episodes of urticaria, in 1966 and during 1971 and 1972. The other patient a woman aged 40, had a chronic recurrent urticaria that began in 1965 and is still active in 1975. She had sarcoidosis confirmed by scalene node biopsy, with hilar adenopathy and a negative tuberculin test between 1969 and 1972. In 1969 she also developed vitiligo. Both patients had a slightly diminished reaction to DNCB sensitization for their age groups, compared to matched controls.[24]

Other laboratory findings

The results of laboratory tests in blood of patients with chronic urticaria, were in general non contributory.

In patients with cold urticaria, no cryoglobulins, cold agglutinins, positive syphilis serology or biphasic cold hemolysins were found.

Eosinophilia was not found except in patients with worm infestations.

Results of different forms of treatment

The main results are combined in table 13. A spontaneous cure was observed in 24 patients between July 1, 1971 and July 1, 1975. The length of the observed asymptomatic period averaged one year, with a range of one half to four years.

TABLE 13. RESULTS OF DIFFERENT FORMS OF TREATMENT IN PATIENTS WITH CHRONIC URTICARIA

	spont remiss	diet asp sensit patients	diet pen sensit patients	treatm periapical granul	treatm worms	antihist control	other	'controlled'*
urticaria factitia (25)	4 (25)	1 (2)	0 (1)	0 (1)	0 (3)	16 (22)		18 (25)
pressure urticaria (17)	1 (17)	0 (7)	0 (2)	0 (0)	0 (3)	0 (17)	prednisone 3(8) tranexamic acid 1(7) phenformin-ethyl-oestrenol 0(12) chloroquin 1	6 (17)
cold urticaria (18)	1 (18)	0 (0)	0 (1)	0 (3)	0 (3)	7 (11)	penicillin 5(10)	10 (18)
cholinergic urticaria (17)	3 (17)	3 (9)	0 (1)	1 (3)	1 (6)	4 (5)	oxyphencyclimine-hydroxyzine 10(15)	12 (17)
heat contact urticaria (1)	0 (1)	0 (1)	0 (0)	0 (0)	0 (0)	1 (1)	heat exhaustion	1 (1)
idiopathic urticaria (56)	13 (56)	9(14)	2 (2)	2(10)	3(10)	12 (23)	pyelonephritis 1	41 (56)
angioneurotic edema (7)	2 (7)	0 (1)	0 (0)	0 (0)	0 (1)	3 (6)	prednisone 1	5 (7)
total (141)	24(141)	13(34)	2 (7)	3(17)	4(26)	43(141)		93(141)

* 'controlled' includes spontaneous remissions and patients controlled by treatment.

A diet free of salicylates, benzoates and azo dyes was effective in 14 of 34 patients, who had been found sensitive to these substances. In this group the follow up period averaged one year, with a range of one half to two years. Some patients noted immediate recurrences with dietary indiscretions, others were able to resume a normal diet after the urticaria had subsided for some time.

A diet free of milk and milk products seemed of benefit to two patients with penicillin hypersensitivity. The result of the dental treatment of periapical granulomas and worm infestation is also noted in table 13, with the restriction that the causal relationship is open to discussion.

Antihistamines were given, if no 'causative' treatment was possible. We used mainly dextrochlorpheniramine maleate, delayed action tablets, 6 to 18 mg per day, or mebhydroline 50 to 150 mg per day, or hydroxyzine dihydrochloride 30 to 75 mg per day. Good results were obtained in urticaria factitia with 16 of 22 patients, especially with hydroxyzine. In about half of the patients with acquired cold urticaria, idiopathic urticaria and angioneurotic edema, the symptoms could be suppressed with antihistamines.

In cholinergic urticaria we used primarily a combination of an anticholinergic drug, oxyphencyclimine 5 to 10 mg per day with hydroxyzine 25 to 75 mg per day. In others antihistamines alone were sometimes effective.

In acquired cold urticaria, penicillin in a dosage of 10 injections of one million units of procain penicillin over a two week period, caused suppression of symptoms in three of six patients for periods of three to twelve months.

Patients with pressure urticaria did not respond to antihistamines, although in some it relieved the accompanying spontaneous urticaria. The effect of a combination of phenformine HCl (phenyl diguanide) and ethyl oestrenolum was tried. This combination has a fibrinolysis enhancing effect.[67] The patients received 50 mg phenformine delayed action and 2 mg ethyl oestrenolum, twice a day by mouth, for periods of one to three months. It was unsuccessful in all of 12 patients who received the drugs.

Tranexamic acid, an inhibitor of plasminogen activation, 1.5 to 3.0 g per day by mouth, for periods of one to three months, was tried in 7 patients. One patient showed a remarkably good result and was able to resume work after two years of illness. The others did not respond.

Prednisone was given to 8 patients with pressure urticaria. In five it

was not possible to find an acceptable maintenance dose. In two patients even 80 mg daily did not control the disease. In three patients dosages of 5 mg per one to three days, controlled the symptoms. Chloroquin was effective in the patient with pressure urticaria and symptoms suggestive of SLE.

In the last column of table 13 under the heading 'controlled', we have combined the patients with spontaneous remissions and the patients whose symptoms could be cured, or controlled by drugs. It shows that a majority of the patients with chronic urticaria is relieved of their symptoms in some way, although one third of the patients continues to suffer.

DISCUSSION

Prevalence and classification

Patients with all forms of urticaria made up 1.4 % of the total number of new patients seen in our department over the three year period of study, which is comparable to the 1–2 % found in most studies.[9,25] The traditional belief that chronic urticaria occurs more often in females,[5,26,27] was not borne out by recent population studies in Sweden.[28] Our finding of about equal numbers of males and females probably is more closely representative of the true prevalence and is confirmed by other recent studies.[4,9,28]

The average age at onset in our group was 28 years, which is in line with other publications.[5,10] The mean duration of 2.3 years compares favourably with the 6 years found by Green *et al.*[10] and Sheffer,[29] but stresses the magnitude of the problem.

We demonstrated the presence of physical urticarias in 55 % of the cases. This is the result of meticulous history taking and persistent follow up of the findings with various test methods. Urbach,[5] who found 20 % physical urticarias among 500 urticaria patients, already realized that he missed many cases, such as some types of cold urticaria that cannot be detected with the exclusive use of the ice cube test. However other recent studies show prevalences similar to ours.[4,30,31]

Urticaria factitia was seen most frequently, followed by equal percentages of cold contact, cholinergic heat and pressure urticaria. Illig *et al.*[32] found in decreasing order of frequency: urticaria factitia, cold contact urticaria, cholinergic heat urticaria, pressure urticaria and light urticaria. Light urticaria did not occur in our series. The two patients

with familial cold urticaria, who were seen during the period of study, were considered to be so different from the other types of physical urticaria that they were treated as an entirely separate group (chapters 4 and 5).

Overlapping of different types of physical urticaria has rarely been described.[32] The combination of pressure urticaria with chronic idiopathic urticaria was seen most frequently and occurred in 15 of our 17 cases. It was noticed by Illig *et al.*[32] in 12 of 14 patients with pressure urticaria, by Rorsman [30] in 14 of 17 and by Ryan *et al.*[33] in 4 of 15 cases. Urticaria factitia coincided with other types of urticaria in a small number of cases in our and Illig's series.[32] We were unable to confirm Illig's finding, of a frequently occurring combination of cholinergic heat urticaria with cold contact urticaria. There was a history suggestive of cold sensitivity in 6 of 17 cases with cholinergic urticaria, but all tests for cold contact urticaria were negative.

Atopy in chronic urticaria

There are few comparable data in the literature, because of a lack of agreement on criteria for atopy. Only studies, in which a control group subject to the same definitions and method was used, can be of value. Champion *et al.*[2] compared 554 urticaria patients and 200 controls. They found no differences in prevalence of atopic diseases in the urticaria patients or their first degree relatives and the controls, on the basis of the history alone. This finding is confirmed by our study (Table 6). However, when we used a more dynamic definition of atopy involving both the history and the patient's skin reactions to a series of well standardized allergens, we found a significantly increased number of atopics in the groups with idiopathic urticaria and urticaria factitia. Aoyama [34] demonstrated that in some patients with urticaria factitia, the sensitivity to trauma could be passively transferred to controls by the Prausnitz-Küstner technique. It would be interesting to know, if these patients are atopics by our definition.

Our study shows that the routine use of a standardized series of intracutaneous tests serves to define those patients who are atopics, but that it rarely helps to find the cause of the urticaria.

The frequency of positive intracutaneous tests in patients with urticaria, has often been blamed on the presence of urticaria factitia. We agree with Grolnick,[35] that this usually does not interfere with the test results.

The non specific delayed wealing reaction, which we noted in 10 of our 17 pressure urticaria patients after intracutaneous tests, drawing of blood or insect bites, has been noticed by several authors.[4,31,33,36] We could not confirm Michaëlsson's suggestion, that this was due to the absence of a kallikrein inhibitor [31] (see chapter 4).

Penicillin allergy

The prevalence of penicillin reactions among urticaria patients ranges from 7 to 24 %,[10,11,31,37] but not in all of these studies intracutaneous tests confirmed the diagnosis. We obtained positive skin tests in three of six patients with a history of penicillin reactions. Skin testing of chronic urticaria patients with penicillin antigens is a valuable aid in diagnosis.

Focal infections

The assignment of a role in the etiology of urticaria to focal infections, dates back to the beginning of this century and has always been highly controversial.[38-40] Kallos [41] gave criteria proving a role for focal infections:

1. A statistically significant difference in the prevalence of focal infections has to be shown, between the groups of study and the controls.
2. The focal infection has to be demonstrated in the patient.
3. The patient has to be shown to be allergic to microorganisms cultured from the focal infection.
4. The illness has to be of an allergic nature.
5. The allergen and the disease have to be proven to have an etiological relationship.

Kallos considered these criteria fulfilled only for certain dermatophytids, tuberculids and syphilids. No such studies exist on chronic urticaria and most authors have considered the demonstration of a focus coinciding with the urticaria and a prompt and permanent cure after treatment of the focus, sufficient proof. However, good results may be due to suggestion or spontaneous cure. Allergies to drugs used against the infection, have to be considered as well. Finally removal of an infection and subsequent subsidence of the urticaria, does not prove a causal relationship.

The incidence of focal infections in patients with urticaria and controls did not show a significant difference in published reports.[25,42,43]

Helgren *et al.*[25] found a slight increase in sinusitis in urticaria patients compared to psoriasis controls, but this was not confirmed by Fikentscher *et al.*[42]

In our material treatment of periapical granulomas only, may have caused a cure of the urticaria in three out of 18 patients.

These results are comparable to those of Resch *et al.*[44] who found dental abnormalities in 50 of 100 urticaria patients, of whom 17 were treated and three showed a prompt and permanent cure. Rorsman [30] found dental abnormalities in 63 of 96 chronic urticaria patients, 50 were operated and two showed a permanent cure.

Neither we nor others, as far as we know, have attempted to culture periapical granulomas from patients with chronic urticaria. It is known[45] that several types of streptococci (hemolytic, indifferent and anaerobic indifferent) can be cultured from some of the periapical granulomas, although most of them are probably not infected. The validity of such studies is hampered by the possibility of contamination with oral flora.[45,46]

In the absence of improved methods of study, it seems rational to continue to treat patients with chronic urticaria for dental abnormalities. This, although subconsciously we may be cautioned by the advice of H. W. Siemens about focal infections: 'immer daran denken, nie daran glauben'.[41]

The evidence for a role of fungus infections in the etiology of urticaria, rests mainly on four isolated case reports[47–50] of patients, who showed a combination of urticaria and fungus infections. In the four patients described in these papers, there were positive skin tests to trichophytin in four, positive fungus cultures in two. Three patients were cured of their urticaria after treatment of the tinea. Three of these patients apparently were atopics and a positive Prausnitz-Küstner reaction with trichophytin was found in one patient.

Apparently an atopic sensitivity to trichophytin exists and it is not unlikely that in some atopic patients a fungus infection of the skin may help maintain the urticaria. Treatment of our five proven cases of fungus infection did not result in a cure of the urticaria. None of the five were atopics however.

Worm infestations

Although chronic urticaria is known as a symptom of some tropical

worm infections, such as filariasis[18] and strongyloidosis,[19] the relationship with Ascaris lumbricoides and Enterobius vermicularis (oxyuris) infections is doubtful. Pasricha *et al.*[51] compared 78 cases of chronic urticaria with 50 cases of other skin diseases and found more gastrointestinal parasites in the control group. Ascaris was seen in 6.4 % and oxyuris in 3.8 % of urticaria patients. Of the 25 patients who were treated, only two with amoebiasis showed improvement. Ascaris migrates in the bloodstream and acute urticaria has been seen in experimental human infections and in laboratory infections.[8,52] There is no evidence however that Ascaris is responsible for chronic urticaria.

The evidence for a role of oxyuris, which is estimated to be present in 20 % of U.S. and British children,[53] is even more doubtful. The only symptom, which has been ascribed to oxyuriasis with certainty sofar, is pruritus ani.[53] Although this parasite resides only in the intestinal tract, it does seem to cause eosinophilia. We noted a prompt and permanent cure of the urticaria in three of 19 patients with oxyuris infections. It seems wise to continue to search for worm infestations in patients with chronic urticaria and to treat them for these infestations, especially since little risk is involved.

Diseases associated with immunological phenomena

Urticaria has been described as a symptom of systemic lupus erythematosus in 6–62 % of cases.[54–56] In the five-year-old girl in our series urticaria antedated the SLE symptoms by one year. We have not found figures in the literature on the prevalence of SLE among urticaria patients. Jansen *et al.*[57] described a patient with urticaria and SLE in whom the urticarial lesions as well as the SLE lesions showed deposits of immunoglobulins. Immunoglobulins were not present in the skin of our young female patient.

Urticaria has not been described in association with Hashimoto's disease.[58] The incidence of vitiligo in our study was 3.5 %. This is within the range of population and hospital population studies found in the literature.[59,60] These figures and the lack of correlation between the onset and localization of urticaria and vitiligo, make a pathogenic relationship unlikely. Two cases of combined pressure urticaria and vitiligo in a father and daughter, have been published.[61]

Urticaria has not been described as a skin manifestation of sarcoidosis.[62,63] Rorsman [30] found one case of sarcoidosis among 106 patients

with severe urticaria. Although deficiencies in cellular immunity are characteristic for sarcoidosis, there is no evidence for increased immediate type hypersensitivity reactions.[62]

Our findings confirm the occurrence of urticaria as an early symptom of SLE. The combinations of urticaria with vitiligo and sarcoidosis are perhaps coincidental.

Results of treatment

In patients not responding to 'causative' treatment, such as avoiding penicillin, azo dyes, and benzoates in the diet or those not responding to the treatment of dental granulomas or worm infestations, antihistamines were given. We have found it useful to try different, chemically not related antihistamines, because of individual differences in response. Hydroxyzine which is marketed as a tranquillizer, is known to be effective in chronic urticaria.[64] It has a strong and long acting antihistamine effect, as illustrated by its suppression of intracutaneous test results with allergens.[65]

Penicillin has been used successfully for the treatment of cold urticaria by several authors.[66] Our limited results seem to confirm this unexplained effect.

Pressure urticaria is notoriously resistant to treatment. Recently a fibrinolysis enhancing combination of two drugs, phenformin and ethyl oestrenolum, was used with some success in pressure urticaria.[67] We were unable to reproduce these results. The use of an antifibrinolytic drug: tranexamic acid, which was of benefit in the hands of others,[68] was successful in one out of our seven patients.

CONCLUSIONS AND SUMMARY

The prevalence of chronic urticaria in a University skin outpatient department over a three year period was 1.1%. In a group of 141 patients with chronic urticaria, with a duration of over three months, representing 76% of the total number of cases, a study of classical pathogenetic factors was done. There was no preponderance of either sex, the average age at onset was 28 years, the average duration at first visit 2.3 years.

Physical urticarias were demonstrated by testing all patients with a number of standardized procedures. A study of the role of atopy and allergic factors was made by history and intracutaneous tests. Focal

infections in particular of teeth and sinuses were detected and treated. Relevant intercurrent diseases such as SLE were evaluated.

Physical urticarias were found in 55 % of cases, considerably more often than the 10–20 % noted in older studies. It confirms the findings of some recent smaller studies. Our findings stress the need to study all patients with chronic urticaria for physical causes, because of the important consequences of such findings for the prognosis and treatment.

In our study atopy was not an important fact in the etiology of urticaria, although it occurred slightly more frequently than in a control group, especially in idiopathic urticaria and urticuria factitia. In only two patients there was a correlation between positive intracutaneous tests, exposure to allergen and clinical symptoms.

Intracutaneous tests with allergens are useful for the detection of atopic factors. However it is unlikely that determination of IgE levels and the use of radio allergo sorbent tests will be of benefit for the diagnosis of chronic urticaria patients, except in selected cases.

Skin tests with penicillin antigens were helpful to detect penicillin allergies, which may cause chronic urticaria. Two patients with chronic urticaria and penicillin allergy were cured of their urticaria, after the use of a diet free of milk and milk products.

No new evidence for a role of focal infections has been produced. Within the limits of this study, it was not possible to exclude a role for focal infections and infestations in seven patients. Of these, three had worm infections, three periapical dental granulomas and one a pyelonephritis. It seems wise to continue to diagnose and treat dental granulomas, worm infestations, fungus infections and other intercurrent infections in patents with chronic urticaria, until improved methods of study are available.

Our study confirmed that urticaria may be an early manifestation of systemic lupus erythematosus.

REFERENCES

1. Wolf-Eisner A: Über die Urticaria vom Standpunkte der neuen Erfahrungen über Empfindlichkeit gegenüber körperfremde Eiweiszsubstanzen. *Dermatol Centralblatt* 10, 164–172, 1907.
2. Champion RH, Roberts SOB, Carpenter RG et al: Urticaria and angio-edema. A review of 554 patients. *Br J Dermatol* 81, 588–597, 1969.
3. Nizami RM, Toyer Baboo M: Office management of patients with urticaria: an analysis of 215 patients. *Ann Allerg* 33, 78–85, 1974.

4. Miller DA, Freeman GL, Akers WA: Chronic urticaria. A clinical study of fifty patients. *Amer J Medic* 44, 68–86, 1968.
5. Urbach E: Kritische Uebersicht über 500 eigene Urticariafälle. Zugleich ein Beitrag zum Problem: allergische oder pathergische Urticaria. *Münch Mediz Wochenschrift* 84, 2054–2060. 1937.
6. Hopkins JG, Kesten BM: Urticaria. Etiologic observations. *Arch Dermatol Syphilol* 29, 358–381, 1934.
7. Sheffer AL: Urticaria and angioedema. *Ped Clin North Am* 22, 193–201, 1975.
8. Gell PH, Coombs RRA: *Clinical aspects of immunology*, ed 2, Oxford, Blackwell Scientific Publications, 1968, pp 583–588.
9. Kleine Natrop HE, Sebastian G: Analyse and Kritik der Urticaria-Diagnostik. *Dermatol Monatsschrift* 159, 769–778, 1973.
10. Green GR, Koelsche GA, Kierland RR: Etiology and pathogenesis of chronic urticaria. *Ann Allerg* 23, 30–36, 1965.
11. Siegel SC, Bergeron JG: Urticaria and angioedema in children and young adults. Etiologic and electrocardiographic findings in one hundred and fifteen cases. *Ann Allerg* 12, 241–252, 1954.
12. Parish WE, Rhodes E: Bacterial antigens and aggregated gamma globulin in the lesions of nodular vasculitis. *Br J Dermatol* 79, 131–147, 1967.
13. Lockshin N, Hurley H: Urticaria as a sign of virus hepatitis. *Arch Dermatol* 105, 481–487, 1964.
14. Ljunggren B, Möller H: Hepatitis presenting as transient urticaria. *Acta Derm Venereol* 51, 295–297, 1971.
15. Weber G, Stetter H: Zur Frage: hautbeschränkte Ornithoseformen. *Hautarzt* 20, 60–63, 1969.
16. Steck WD, Byrd RB: Urticaria secondary to pulmonary melioidosis. Report of a case. *Arch Dermatol* 99, 80–81, 1969.
17. Singh G, Ojha D: Chronic urticaria and filariasis. *Dermatologica* 136, 173–175, 1968.
18. Doeglas HMG, Ten Berg JAG: Larva currens (migrans) caused by Strongyloides stercoralis. *Dermatologica* 144, 350–352, 1972.
19. Kissin M, Adleman RJ: Transient urticaria in malaria. *Am J Trop Med Hyg* 28, 797–802, 1948.
20. Braverman IM: Urticaria as a sign of internal disease. *Postgrad Med* 41, 450–454, 1967.
21. Bleumink E: *Isolation and characterization of some atopic food allergens.* Thesis. Utrecht 1967.
22. Faust EC, Sawitz W, Tobie J et al: Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. *J Parasitol* 25, 241–262, 1939.
23. Ritchie LS: An ether sedimentation technique for routine stool examinations. *Bull US Army Med Department* 8, 326, 1948.
24. Bleumink E, Nater JP, Schraffordt Koops H et al: A standard method for sensitization testing in patients with neoplasms. *Cancer* 33, 911–915, 1974.
25. Helgren L, Hersle K: Acute and chronic urticaria. A statistical investigation on clinical and laboratory data in 1204 patients and matched healthy controls. *Acta Allergol* 19, 406–420, 1964.
26. Castelain PY, Bonniol P, Saint André P et al: Nouvelles recherches sur l'étiologie et la thérapeutique des urticaires chroniques. A propos de 100 observations. *Bull Soc Fr Dermatol Syphiligr* 78, 578–584, 1971.
27. Calnan CD: Urticarial reactions. *Br. Med J* 2, 649–655, 1964.
28. Helgren L: The prevalence of urticaria in the total population. *Acta Allergol* 27, 236–240, 1972.

29. Sheffer AL: Urticaria and angioedema. *NY State J Med* 72, 922–928, 1972.
30. Rorsman H: Basophilic leucopenia in different forms of urticaria. *Acta Allergol* 17, 168–184, 1962.
31. Michaëlsson G: Chronic urticaria. A clinical study with special reference to vascular reactions mediated by the kallikrein-kinin system. *Acta Derm Venereol* 49, 404–416, 1969.
32. Illig L, Kunick J: Klinik und Diagnostik der physikalischen Urticaria. I. *Hautarzt* 20, 167–178, 1969.
33. Ryan TJ, Shim Young N, Turk JL: Delayed pressure urticaria. *Br J Dermatol* 80, 485–490, 1968.
34. Aoyama H: IgE as a dermographism inducing principle of urticaria factitia. *Jap J Dermatol*, Series B, 81, 266–271, 1971.
35. Grolnick M: An investigative and clinical evaluation of dermographism. *Ann Allerg* 28, 395–404, 1970.
36. Kalz F, Bower CM, Prichard H: Delayed and persistent dermographia. *Arch Dermatol Syphilol* 61, 772–780, 1950.
37. Tas J: Chronic urticaria. A survey of one hundred hospitalized cases. *Dermatologica* 135, 90–96, 1967.
38. Lustgarten: Urticaria and toxaemic conditions. *J Cutaneous Diseases* 25, 216–217, 1907.
39. Barber HW: Chronic urticaria and angio-neurotic edema due to bacterial sensitization. *Br J Dermatol Syphilol* 35, 209–218, 1923.
40. Leriche R: Guérison définitive d'urticaire à type anaphylaxique par l'appendicectomie. *Presse Med* 44, 916–917, 1936.
41. Epstein St, Halter K, Kallos P, Lutz W, Marchionini A, Tappeiner S, Siemens HW: Für welche Dermatosen sind Fokalinfektionen bedeutsam? *Derm Wochenschr* 123, 99–109, 1951.
42. Fikentscher R, Koester H, Spinar H: Die routinemäszige Fokussuche bei Hauterkrankungen und ihre Trefferquote im HNO Bereich. *Derm Monatsschr* 157, 564–569, 1971.
43. Schmidl M, Kern A: Zur Pathogenese der Urticaria chronica. *Derm Wochenschr* 150, 481–487, 1964.
44. Resch CE, Evans RE: Chronic urticaria and dental infection. *Cleve Clin Q* 25, 147–150, 1958.
45. Winkler KC, Amerongen J van: Bacteriologic results from 4000 rootcanal cultures. *Oral Surg* 12, 857–875, 1959.
46. Wijk PH: *Behandeling van non vitale pulpa met formocresol.* Thesis, Leiden, Stafleu en Tholen, 1972.
47. Wise F, Sulzberger MB: Urticaria due to Trichophyton (Epidermophyton interdigitale). *JAMA* 95, 1504, 1930.
48. Waldbott GL, Ascher MS: Chronic urticaria recurring every six weeks due to a fungus infection. *Arch Dermatol Syphilol* 36, 314–317, 1937.
49. Shelley WB, Florence R: Chronic urticaria due to mold hypersensitivity. A study in cross sensitization and autoerythrocyte sensitization. *Arch Dermatol* 83, 549–558, 1961.
50. Weary PE, Guerrant JL: Chronic urticaria in association with dermatophytosis. *Arch Dermatol* 95, 400–401, 1967.
51. Pasricha JS, Pasricha A, Prakash OM: Role of gastrointestinal parasites in urticaria. *Ann Allerg* 30, 348–351, 1972.
52. Phills JA, Harrold JA, Whiteman GV et al: Pulmonary infiltrates, asthma and eosinophilia due to Ascaris Suum infestation in man. *N Engl J Med* 286, 965–970, 1972.

53. Editorial: Children's worms. *Br Med J* 4, 3, 1974.
54. Harvey AM, Shulman LE, Tumulty PA et al: Systemic lupus erythematosus. *Medicine* 33, 291–437. 1954.
55. Dubois EL: *Lupus erythematosus*. New York, Mc Graw Hill Book Company, 1966.
56. Scott A, Rees EG: The relationship of systemic lupus erythematosus to discoid lupus erythematosus. *Arch Dermatol* 79, 422–435, 1959.
57. Jansen LH, Cormane RH: Urticaria bij lupus erythematodes. *NedTijdschr Geneeskd* 111, 2160–2165, 1967.
58. Mulhern LM, Masi AT, Shulman LE: Hashimoto's disease. A search for associated disorders in 170 clinically detected cases. *Lancet* 2, 508–511, 1966.
59. Helgren L: *An epidemiological survey of skin diseases, tattooing and rheumatic diseases*. Stockholm, Almquist and Wiksell, 1967.
60. Grunnet I, Howitz J, Reymann F et al: Vitiligo and pernicious anemia. *Arch Dermatol* 101, 82–85, 1970.
61. Flechs K: Vitiligo und Druck-Urticaria. *Z Haut Geschlechtskr* 45, 680–681, 1970.
62. Scadding JG: *Sarcoidosis*. London, Eyre and Spottiswoode, 1967, pp 174–194.
63. Rook A, Wilkinson DS, Ebling FJG: Sarcoidosis, in: *Textbook of Dermatology*, ed 2, Oxford, Blackwell Scientific Publications, 1972, vol 2, pp 1412–1436.
64. Feinberg AR, Pruzansky JJ, Feinberg SM et al: Hydroxyzine (Atarax) in chronic urticaria and in allergic manifestations. *J Allergy* 29, 358–361, 1958.
65. Galant SP, Bullock J, Wong D et al: The inhibitory effect of anti-allergy drugs on allergen and histamine induced wheal and flare response. *J Allergy Clin Immunol* 51, 11–21, 1973.
66. Liebeskind H, Schwarze G: Zur Problematik der Penicillintherapie der Kältekontakturticaria. *Hautarzt* 25, 482–485, 1974.
67. Ryan TJ, Nishioka K, Dawber RPR: Epithelial-endothelial interaction in the control of inflammation through fibrinolysis. *Br J Dermatol* 84, 501–515, 1971.
68. Michaëlsson G: personal communication. 1974.

CHAPTER 2

REACTIONS TO ASPIRIN AND FOOD ADDITIVES IN PATIENTS WITH CHRONIC URTICARIA, INCLUDING THE PHYSICAL URTICARIAS*

H. M. G. DOEGLAS

INTRODUCTION

It has been shown that aspirin is an important eliciting factor in a considerable percentage of patients with chronic idiopathic urticaria and asthma.[1-4]

So far only one group of investigators has tested patients with physical urticarias for aspirin sensitivity, with negative results.[1] In aspirin sensitive patients with chronic urticaria and asthma, reactions have also been described to certain food additives and analgesics.[3,5] During an investigation of the pathogenesis of chronic urticaria, it was noticed that a history of aspirin sensitivity was present in some patients with physical urticarias as well as in those with chronic idiopathic urticaria. Therefore it was decided to perform aspirin provocation tests in a group of patients with chronic urticaria, including many patients with physical urticarias. Patients with positive aspirin provocation tests were also tested with tartrazine, benzoates, salicylates and the analgesics indomethacin, paracetamol and mefenamic acid.

All urticaria patients were screened for the presence of nasal polyposis sinusitis, asthma and atopy.

PATIENTS, MATERIALS AND METHODS

Patients

A group of 131 patients with urticaria of more than 3 months duration, observed over a 3 year period, was tested. All had been studied intensively for the presence of physical urticarias. The criteria for diagnosis and the tests used have been described[6] (See chapter 3).

Included were patients with urticaria factitia, delayed pressure urticaria,

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TABLE 1. TYPE OF URTICARIA AND RESULTS OF PROVOCATION TESTS AMONG PATIENTS WITH CHRONIC URTICARIA

type of urticaria	N	percentage	provocation test positive
physical urticarias			
urticaria factitia	22	16.8	3 (14 %)
pressure urticaria	16	12.2	7 (43 %)
cold urticaria	18	13.7	0
cholinergic urticaria	17	12.9	9 (52 %)
heat contact urticaria	1	0.7	1
total	74	56.4	20 (27 %)
chronic idiopathic urticaria	50	38.1	16 (32 %)
angio edema (acq)	7	5.4	1 (14 %)
total	131	100.0	37 (26 %)

cold urticaria, cholinergic urticaria, patients without physical urticaria (chronic idiopathic) and acquired angio edema (Table 1).

Provocation tests were done during a phase of minimal or absent urticaria. Antihistamines and corticosteroids were discontinued for at least a week before the test. Most patients were studied as outpatients.

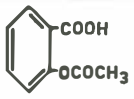
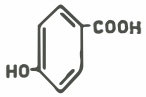
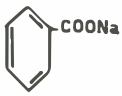
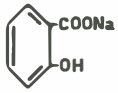
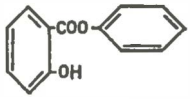
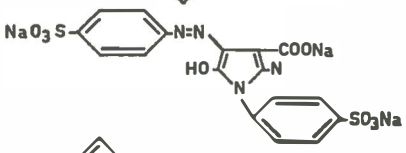
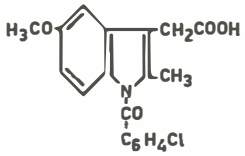
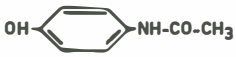
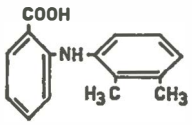
Chemicals used for provocation tests

The dosages and structural formulae are shown in Table 2. They were administered in gelatin capsules with lactose added as filling.

Test method

Urticarial lesions present before testing were marked with a skin pencil. Aspirin was given first. Patients with a history of aspirin sensitivity, asthma, nasal polyposis, recurring sinusitis or rhinitis were given the lowest dosage of aspirin (2 mg). The dosage was increased each hour until symptoms occurred. Patients with a negative history received 1000 mg at once. The patients were observed for 2 h and after 24 and 48 h. Reactions occurring within 2 h were observed, until symptoms had subsided. Doubtful reactions were evaluated in comparison with the placebo or the test was repeated at a later data. After a positive reaction no further tests were done for a week. No more than one substance was tested on each day. Only those patients who showed a reaction to aspirin were tested with the other chemicals as indicated in Table 2.

TABLE 2. DOSAGES AND STRUCTURE OF CHEMICALS USED FOR PROVOCATION TESTS IN PATIENTS WITH CHRONIC URTICARIA

chemical	dosage (mg)	structural formula
aspirin	2, 5, 10, 50, 100 250, 500, 1000	
4-hydroxybenzoic acid	50, 100	
sodium benzoate	250, 500	
sodium salicylate	500, 1000	
phenyl salicylate	500, 1000	
tartrazine	2, 5, 10	
indomethacin	25, 50	
paracetamol	500	
mefenamic acid	250	
lactose placebo	300	

Evaluation of reactions

A provocation test was considered positive, if the patients developed

urticaria or angio edema after it had been absent or if a dramatic increase occurred in the lesions already present, with or without additional symptoms. Additional symptoms observed were: a scarlet flush beginning in the scalp and spreading over the face and chest, conjunctival injection, increased tear secretion, nasal congestion, watery rhinorrhea, cough, hoarseness, wheezing and dyspnea or asthma.

Severe reactions all occurred within 2 h, delayed symptoms might occur up to 24 h.

Nasal polyposis, recurring sinusitis

A careful search for these symptoms was made in the history and by examination in the E.N.T. department. Roentgenograms of the paranasal sinuses were made when there was the slightest suspicion of pathology present.

Presence or absence of atopy

Patients were classified as atopics, if they had a positive reaction to one or more of 3 intracutaneous skin tests with house dust (0.1 %), human dander (0.1 %) or animal dander (0.01 %) (Haarlem Allergen Laboratory, the Netherlands), together with evidence of asthma, seasonal hay fever or atopic dermatitis in the patient or his first degree relatives. Histamine (i.c.) 0.1, 0.01, 0.001 mg/ml was used as a standard for the skin tests. The results in the aspirin positive and negative urticaria groups were compared with those of a control group of skin disease patients from the same clinic without atopic dermatitis. The control group was matched for sex and age and was questioned and tested specifically for this purpose.

Diet free of tartrazine, benzoates and salicylates

In the Netherlands the use of food additives is regulated by law. All foods in which the law permitted the use of tartrazine or benzoates were forbidden. Fruits known to contain salicylates were also eliminated.

Determination of protease inhibitors and complement values in plasma

Determinations of anti-trypsin, anti-chymotrypsin and anti-kallikrein activity of plasma of aspirin sensitive patients and controls were performed according to methods described in earlier papers[6,7] (see chapter 3).

Measurements of levels of C1 esterase inhibitor, α_1-antitrypsin and α_2-macroglobulin and the complement factors C3 and C4 were described in the same papers. Micro hemolytic complement titration of plasma was performed after Levine.[8] The data are recorded as CH50 values or as the reciprocal of the amount in ml in which 50% of 5×10^7 sensitized sheep erythrocytes will lyse in 1 h incubation at 37° C.

RESULTS

The group of 131 patients consisted for 57 % of patients with physical urticarias (Table 1). This high percentage probably reflects the type of urticaria patient referred to a University clinic, but is also a result of an active search for physical causes.[6]

In thirty seven of 131 patients (26 %), a positive provocation test with aspirin was observed (Table 1). There were notable differences in the incidence of aspirin sensitivity in the subgroups. The highest incidence (52 %) was in the patients with cholinergic urticaria, followed by 43 % in the group with delayed pressure urticaria and 32 % in the group with chronic idiopathic urticaria. Lower incidences were observed in the patients with urticaria factitia and acquired angio edema, while none of the patients with cold urticaria showed positive reactions.

One patient with contact heat urticaria had marked angio edema after aspirin administration. There were thirteen females and twenty four males in the aspirin positive group and fifty four females and forty males in the aspirin negative group. The sex distribution in the various types of urticaria was unremarkable.

The type of skin symptoms noticed in the provocation tests consisted of urticaria in thirty six patients, angio edema in fifteen and a very typical scarlet flush beginning in the scalp and spreading downward to the shoulders and chest in eight patients. In nine patients there were eye symptoms such as injection of the conjunctiva, increased tear secretion and burning; in seven patients nasal congestion, sneezing and a running nose were observed; four complained of an irritable cough, four had swelling of the throat or hoarseness and nine complained of various degrees of dyspnea. In individual patients tachycardia, gastric hyperacidity and pain, fever and purpura occurred. These additional symptoms were seen predominantly in the patients with cholinergic and with chronic idiopathic urticaria.

There was evidence of previous aspirin reactions in the history of only

ten patients, five had noted swelling of the eyelids or lips and two patients with cholinergic urticaria had suffered a severe reaction with systemic symptoms. Among these ten patients the reaction could be reproduced by provocation test in seven. In thirty patients without a history of aspirin reactions, the test was also positive; nine of these patients claimed never to have used aspirin containing drugs before.

The question of a quantitative effect of aspirin was studied in nine patients who received graduated dosages. The lowest dosages to which they responded were 50 mg in two, 100 mg in one, 250 mg in three, 500 mg in one and 1000 mg in two patients. In four of these patients the

TABLE 3. PROVOCATION TESTS WITH OTHER CHEMICALS IN ASPIRIN SENSITIVE PATIENTS WITH CHRONIC URTICARIA

type of urticaria	patient no	sex	tart	4-OH benz	sod benz	sod sali	phenyl sali	indo meth	para cet	mefen acid
urticaria factitia	1	F	−	−	−	−	+	−	−	−
pressure urticaria	2	M	+	−	+	−	−	−	−	−
	3	F	−	−	−	+	+	−	−	−
	4	M	−	−	−	−	−	−	−	+
cholinergic urticaria	5	M	+	−	−	−	−	+	−	−
	6	M	−	−	−	−	−	+	−	−
	6	F	−	−	+	0	0	−	−	−
	8	M	+	−	−	−	−	0	0	0
	9	F	−	−	−	−	−	−	−	−
chronic idiopathie urticaria	10	F	+	+	+	+	−	+	−	+
	11	F	+	−	−	+	−	+	+	−
	12	F	−	+	+	−	−	+	−	−
	13	M	−	−	−	+	+	+	−	−
	14	F	+	+	−	0	0	+	−	−
	15	F	−	+	−	−	−	−	−	−
	16	M	−	−	+	−	−	−	−	−
	17	M	−	−	−	+	0	0	0	0
	18	M	−	−	−	−	+	−	−	−
	19	M	−	−	−	−	−	+	−	−
	20	F	−	−	−	−	−	−	−	−
	21	M	−	−	−	−	−	−	−	−
	22	M	−	−	0	0	0	0	0	0
angio edema (acq)	23	F	+	−	−	+	+	+	−	+
positive/tested			7/23	4/23	5/22	6/20	5/19	9/20	1/20	3/20
percentage			30.4	17.4	22.7	30.0	26.3	45.0	5.0	15.0

response was doubtful at these dosages and they produced a more clear-cut reaction on the next higher dose. In one patient 500 mg was given after a slight erythema of the scalp had been produced with 50 mg. He developed the most severe reaction which we have seen, with lacrimation, running nose, flush, urticaria, angio edema and an asthma attack. After the dosages of 100 and 250 mg had been introduced, such severe reactions have not been seen anymore.

The interval between ingestion and symptoms was variable. In thirty-two positive aspirin provocation tests the interval was less than 2 h; this included the only two severe reactions which were seen. In twenty-two tests it was less than 12 h and in two instances between 12 and 24 h.

The duration of the symptoms induced by the provocation tests was usually from a few to 24 h. In some patients exacerbations of the urticaria were induced lasting a week.

For reasons of expedience, tests with the other chemicals could be performed in only twenty three of the thirty seven aspirin sensitive patients (Table 3). There was no correlation between any particular type of urticaria and a reaction to a specific chemical.

The highest incidence of reactions was found to indomethacin, to which nine of twenty (45 %) patients responded, whereas seven of twenty three (30.4 %) reacted to tartrazine, six of twenty (30 %) to sodium salicylate, five of nineteen (26.3 %) to phenyl salicylate, five of twenty two (22.7 %) to sodium benzoate, four of twenty three (17.4 %) to 4-hydroxy benzoic, acid, three of twenty (15 %) to mefenamic acid and one of twenty (5 %) to paracetamol. Only one of the nine patients responding to indomethacin had ever used the drug before, only one of four had ever used paracetamol and none had used mefenamic acid.

The incidence of nasal polyposis and asthma (Table 4) did not differ significantly between the aspirin positive and negative groups. Sinusitis occurred more often in the aspirin positive group.

TABLE 4. ASPIRIN SENSITIVITY IN CHRONIC URTICARIA PATIENTS RELATED TO NASAL POLYPOSIS, SINUSITIS AND ASTHMA

	aspirin provoc negative	aspirin provoc positive	total	significance
	91	33	124	
polyposis	4 (4.4 %)	3 (9.1 %)	7 (5.6 %)	n.s.
sinusitis	1 (1.1 %)	3 (9.1 %)	4 (3.2 %)	$p = 0.02–0.05$
asthma	6 (6.6 %)	1 (3.0 %)	7 (5.6 %)	n.s.

Among ninety four patients with chronic urticaria and a negative aspirin provocation test, there were nineteen who could be classified as atopics (22.1 %). In the aspirin positive group there were four of thirty-seven (10.8 %) and in the control group three of fifty (6.0 %).

The differences between the aspirin positive and negative groups are statistically not significant. Atopics did occur significantly more often in the aspirin negative group compared to the controls.

Patients sensitive to aspirin received a diet free from tartrazine, benzoates and salicylates. The diet was used by twenty seven patients. The results were good to excellent in seventeen of them during a follow-up period of 1–8 months.

Several patients noted exacerbations after the use of forbidden foods. It was also noticed that after the disappearance of the urticaria, some of the patients were able to resume a normal diet without recurrence of the symptoms.

In a previous study[6] (see chapter 3), a number of protease inhibitors and the complement values C3 and C4 were determined in the plasma of ninety two patients with chronic urticaria and a control group of patients with other skin diseases. The same determinations were performed in the plasma of twenty one patients with aspirin sensitive urticaria and compared with a control group of sixty eight patients with other skin diseases. There were no significant differences for the levels of protease inhibitors (Table

TABLE 5. PROTEASE INHIBITORS AND COMPLEMENT FACTORS IN PATIENTS WITH ASPIRIN HYPERSENSITIVITY

	N	C1 esterase inhibition	trypsin inhibition	chymo-trypsin inhibition	kallikrein inhibition	α_1-anti-trypsin	α_2-macro-globulin
patients with aspirin hyper-sensitivity	21	0.99±0.32	1.369±0.281	0.787±0.271	0.146±0.044	313±93 (n=20)	222±73 (n=20)
controls	68	0.86±0.28	1.251±0.266	0.701±0.332	0.139±0.033	273±72	268±83

(no significant differences, Student *t* test)

5), and in particular the values of C3, C4 and total hemolytic complement (Table 6), were normal.

In seventeen plasmas, collected during the acute phase of aspirin reac-

tions after aspirin provocation tests, the values for C3, C4 and total hemolytic complement did not show deviations from the control group (Table 6).

TABLE 6. COMPLEMENT FACTORS C3 AND C4 AND TOTAL HEMOLYTIC COMPLEMENT IN ASPIRIN SENSITIVE PATIENTS AND CONTROLS

	N	C3	C4	hemolytic complement*
patients in quiescent phase	21	87±18	37.6±10.1	not done
patients in active phase after provocation	17	81±10	29.2± 7.4	116±31 (n = 12)
controls	68	80±14	32.5±10.7	98±23 (n = 34)

* micro complement titration: CH50 values of 5×10^7 cells.

DISCUSSION

The reported incidence of aspirin sensitivity in chronic idiopathic urticaria is between 21 and 75 %[1-3] and between 2 and 10 % in asthma.[4,9] Provocation tests with aspirin, food additives and analgesics have been carried out in urticaria patients and in asthmatics.[3,5,10] These tests should be carried out only under strict supervision in a hospital environment. In our patients with chronic idiopathic urticaria the incidence of aspirin sensitivity was 32 %, which agrees well with published findings. Our study included a large number of patients with physical urticarias. An unexpected finding was the high incidence of aspirin sensitivity among patients with cholinergic urticaria and pressure urticaria. Moore Robinson & Warin [1] studied the effect of 300–1200 mg of aspirin on the outcome of tests for physical urticaria in ten patients with dermographism, three with cold urticaria and five with cholinergic urticaria and found no difference in the reaction to the test before or after aspirin. Moreover, there was no urticarial eruption reported after the use of aspirin alone. The small number of cases with cholinergic urticaria in their series may have obscured the aspirin sensitivity. In the case of pressure urticaria four of the seven aspirin responsive patients also had spontaneous weals, which may have influenced the results. Michaëlsson & Juhlin [3] excluded patients with physical urticarias from their study.

The quantitative effect of aspirin in aspirin sensitive patients noticed

by Moore Robinson & Warin [1] was confirmed in four of our patients who received graded dosages.

The incidence of sensitivity to tartrazine and benzoates was lower in this study than in that of Michaëlsson & Juhlin.[3] This may be explained in part by our use of aspirin as a screening agent, whereas sensitivity to tartrazine and benzoates may occur without aspirin sensitivity.

The finding that only one of nine indomethacin sensitive patients, one of four paracetamol sensitive and none of two mefenamic acid sensitive patients had ever used the drug before, makes a previous allergic sensitization questionable. Samter & Beers [11] also reported indomethacin sensitivity in aspirin sensitive asthmatics who had never used the drug. Smith [5] described sensitivity to paracetamol and mefenamic acid in aspirin sensitive asthmatics.

Reports on sensitization to sodium- and phenyl salicylate are conflicting. Our results show that at least some aspirin sensitive patients do not tolerate them, confirming the findings of Moore Robinson & Warin [1] who noticed worsening of urticaria in thirteen of eighteen aspirin sensitive patients with sodium salicylate and in two of three after phenyl salicylate. Samter saw no reaction after sodium salicylate in forty aspirin sensitive asthmatics, whereas Storm van Leeuwen [9] and James [12] noticed reactions with sodium salicylate in aspirin sensitive asthmatics, although relatively higher dosages were needed. The role of salicylates in foods is probably limited since they are no longer used as additives in most countries and they occur only in small quantities in natural foods.[3]

Aspirin sensitivity in asthmatics occurs especially in those who have nasal polyposis and sinusitis.[10,14,15] This combination has been called the asthma triad.[11] An incidence of 48–73 % of polyposis and sinusitis in aspirin sensitive asthmatics has been reported.[4,10,14]

The search for polyposis and asthma in our urticaria patients did not reveal a significant correlation with aspirin sensitivity. Only one patient had the 'asthma triad' as well as his urticaria. Sinusitis was slightly more frequent in the patients with aspirin reactions, but the evidence is based on a rather small number of cases.

Aspirin sensitivity in asthma patients is found mostly in those with the intrinsic, non-atopic type of asthma with predominantly negative skin tests to inhalants.[11,15,16] There was no evidence of a decreased number of atopics in our aspirin sensitive urticaria patients compared with the other urticaria patients or the controls. Apparently aspirin sensitive urticaria

patients and asthmatics differ in the incidence of polyposis and sinusitis as well as in their relation to atopy.

Little can be said about the mechanism of action of aspirin in aspirin sensitive urticaria patients. Many studies that tried to implicate allergic mechanisms were largely unsuccessful.[14,17,18] The quantitative effect of aspirin, the cross reactivity with chemically non related drugs, the fact that previous sensitization can usually be excluded and the disappearance of the aspirin sensitivity after regression of the urticaria, are inconsistent with an allergic sensitization. The only finding which aspirin, indomethacin, paracetamol and sodium salicylate have in common, is their inhibitory effect on the synthesis of prostaglandins PGE_1 and $PGF_{2\alpha}$.[19] These two mediators have opposed effects on vascular permeability and bronchial muscle.[19,20]

Further study of prostaglandin metabolism might lead to a better insight in the pathophysiology of aspirin induced urticaria.

The finding of a high incidence of aspirin sensitivity in cholinergic pressure and chronic idiopathic urticaria suggests perhaps a common denominator in the etiology of these diseases.

Yurchak *et al.*[17] suggested that a study of complement levels during acute reactions to aspirin seemed worthwhile. We have determined the plasma levels of C3 and C4 and total complement of twenty one aspirin sensitive patients in a quiescent phase and seventeen plasma samples obtained during the acute phase of aspirin reactions, after aspirin provocation tests. There were no deviations from the values of a control group. This suggests that major changes in the complement system are not present during the acute phase of aspirin reactions. Definite proof can be given only when all the individual components of complement are measured.

SUMMARY

In 131 patients with chronic urticaria, including physical urticarias, oral provocation tests were done with aspirin. A total of thirty one patients showed a reaction on aspirin challenge. Reactions were seen in 35 % of patients with idiopathic urticaria, 52 % of patients with cholinergic urticaria and 43 % of those with pressure urticaria.

The patients with reactions to aspirin were also tested with tartrazine, sodium benzoate, 4-hydroxy benzoic acid, sodium- and phenyl salicylate and the analgesics indomethacin, paracetamol and mefenamic acid. In

nineteen of twenty three aspirin sensitive patients, positive reactions to one or more of these substances were observed. Indomethacin and tartrazine had the highest scores. There was no statistically significant correlation between aspirin reactions and the presence of nasal polyposis sinusitis, asthma or atopy.

REFERENCES

1. Moore Robinson M, Warin RP: Effect of salicylates in urticaria. *Br Med J* 4, 262–264, 1967.
2. Champion RH, Roberts SOB, Carpenter RG et al: Urticaria and angio-edema. A review of 554 patients. *Br J Dermatol* 81, 588–597, 1969.
3. Michaëlsson G, Juhlin L: Urticaria induced by preservatives and dye additives in food and drugs. *Br J Dermatol* 88, 525–532, 1973.
4 Settipane GA, Chafee FH: Aspirin intolerance II. A prospective study in an atopic and normal population. *J Allergy Clin Immunology* 53, 200–204, 1974.
5. Smith AP: Response of aspirin allergic patients to challenge by some analgesics in common use. *Br Med J* 1, 494–496, 1971.
6. Doeglas HMG, Bleumink E: Protease inhibitors in plasma of patients with chronic urticaria. *Arch Dermatol*, 111, 979–985, 1975.
7. Doeglas HMG, Bleumink E: A kindred with familial cold urticaria. Clinical findings. *Arch Dermatol* 110, 382–388, 1974.
8. Levine L: Microcomplement fixation, in: Weir DM (ed): *Handbook of Eperimental Immunology*, Oxford, Blackwell Scientific Publications, 1967, pp 707–719.
9. Storm van Leeuwen W: Pathognomonische Bedeutung der Ueberempfindlichkeit gegen Aspirin bei Asthmatikern. *Münch Med Wochenschr* 75, 1588–1590, 1928.
10. Mc Donald JR, Mathison DA, Stevenson DD: Aspirin intolerance in asthma. *J Allergy Clin Immunology* 50, 198–207, 1972.
11. Samter M, Beers RF: Intolerance to aspirin. Clinical studies and consideration of its pathogenesis. *Ann Intern Med* 68, 975–983, 1968.
12. James J: Personal communication, in: Warin RP, Champion RH: Urticaria. *Major problems in Dermatology*, vol. 1, London, England, WB Saunders Company Ltd, 1974, pp 1–173.
13. Juhlin L, Michaëlsson G: Förbjudet och tillåtet vid överkänslighet för konserveringsmedel och färgämnen. *Läkartidningen*, 70, 1414–1416, 1973.
14. Giraldo B, Blumentdhal MN, Spink WW: Aspirin intolerance and asthma, a clinical and immunological study. *Ann Intern Med* 71, 479–496, 1969.
15. Falliers CJ: Aspirin and subtypes of asthma: Risk factor analysis. *J Allergy Clin Immunol* 52, 141–147, 1973.
16. Chafee FH, Settipane GA: Aspirin intolerance. I. Frequency in an allergic population. *J Allergy Clin Immunol* 53, 193–199, 1974.
17. Yurchak AM, Wicher K, Arbesman CE: Immunologic studies on aspirin. *J Allergy* 46, 245–253, 1970.
18. De Weck AL: Immunological effects of aspirin anhydride, a contaminant of commercial acetylsalicylic acid preparations. *Int Arch Allergy Appl Immunol* 41, 393–406, 1971.
19. Collier HOJ: Prostaglandins and aspirin. *Nature* 232, 17–19, 1971.
20. Willoughby DA: Effects of prostaglandin $PGF_{2\alpha}$, and PGE_1 on vascular permeability. *J Pathol Bacteriol* 96, 381–386, 1968.

CHAPTER 3

PROTEASE INHIBITORS IN PLASMA OF PATIENTS WITH CHRONIC URTICARIA*

H. M. G. DOEGLAS, E. BLEUMINK

INTRODUCTION

It has been postulated by investigators such as Duck *et al.*,[1] and Juhlin and Michaëlsson[2], that protease inhibitor deficiencies might play a role role in the pathogenesis of chronic urticaria. These protease inhibitors are present in various tissues and in blood. They are capable of forming complexes with proteases that then become enzymatically inactive. They are able to inactivate C1 esterase, thrombin, plasmin, Hageman factor, and kallikrein, respectively enzymes of the complement, fibrinolysis clotting, and kallikrein systems (Table 1). These inhibitors also inactivate proteolytic enzymes like trypsin, chymotrypsin and lysosomal enzymes (Table 1). When these inhibitors are absent or deficient, the proteases are not inactivated in time and will in turn be capable of activating the mediator systems. As a consequence, the possibility exists that large amounts of pharmacologically active components such as kinins and anaphylatoxins may be released. An example of a skin disease caused by the absence or inactivity of a protease inhibitor is hereditary angioneurotic edema (HEAE). Patients with this disease have a deficiency of the inhibitor of C1 esterase, the activated first component of complement that has protease activity.[3,4] The symptoms are thought to be caused by C2 peptide, a compound with kinin like properties derived from C2 by the action of C1 esterase.[3,4]

The purpose of this investigation was to evaluate the hypothesis of Duck *et al.* and Juhlin and Michaëlsson. Since there are many inhibitors with a wide range of overlapping activities, a number of inhibitors were measured, including α_1-antitrypsin, α_2-macroglobulin, CI-esterase inhibitor, and the inhibitor capacities of plasma for the enzymes trypsin, α-chymotrypsin and urinary kallikrein. These are the major inhibitors

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TABLE 1. PROTEASE INHIBITORS AND SITE OF ACTION IN MEDIATOR SYSTEMS

inhibitor	site of action
α_1-antitrypsin	trypsin chymotrypsin plasmin thrombin kallikrein leukocyte proteases tissue proteases (elastase, collagenase)
α_2-macroglobulin	plasmin kallikrein thrombin trypsin
C1-esterase inhibitor	C1-esterase, activated C1r plasmin kallikrein activated Hageman factor thromboplastin antecedent
kallikrein inhibition capacity	kallikrein
trypsin inhibition capacity	trypsin plasmin thrombin kallikrein leukocyte proteases tissue proteases (elastase, collagenase)
chymotrypsin inhibition capacity	chymotrypsin leukocyte proteases

capable of inhibiting many enzymes in the complement, clotting-fibrinolysis and kallikrein systems and of lysosomal enzymes and tissue proteinases (Table 1).[5-10]

Because deficiencies in C1 esterase inhibitor levels in HEAE patients also entail changes in complement factor levels (C4), it was decided to measure C3 and C4 as well.

PATIENTS AND METHODS

Patients and Controls

A total of 92 patients with urticaria of more than three months duration

were studied. They were seen as outpatients in a University Hospital skin department. All patients were investigated to detect the presence of physical urticarias. Spontaneous lesions, not induced by physical factors, were present in some patients with urticaria factitia and cholinergic urticaria and in most patients with pressure urticaria. Combinations of two or more types of physical urticaria did occur rarely; they were classified under the predominating cause.

Urticaria Factitia (17 patients). All patients were questioned for evidence of rapid onset of lesions after superficial trauma such as rubbing or scratching. All 92 patients were tested, and the results were compared with those of a control group of patients with other skin diseases, matched for sex and age, from the same clinic.

The test consisted of drawing knitting needles with 100, 200, 300, 400 and 500 gm weights over the skin of the upper part of the back, at a 45° angle, through a metal slot. The width of the erythema and edema were measured after 1, 5, and 20 minutes (modified after Grolnick [11]).

Patients with urticaria factitia were defined as those having a history of weal formation after superficial trauma, together with a weal 1 to 2 mm in width when weights of 100 and 200 gm were used. In patients with other types of urticaria and in the controls, the history was negative and there was no weal formation when weights of 100 and 200 gm were tested. Some showed a 1 to 2 mm weal, at the most, with the heavier weights. Pressure tests gave negative findings in all these patients.

Delayed Pressure Urticaria (12 patients). All patients were asked for evidence of the delayed onset of edema and urticaria after heavy pressure especially after manual labor or carrying weights. Tests were performed in 49 patients, including all of those with a history of onset or aggravation after trauma. Only patients responding to the test were included.

A weight of 8.000 gm was hung on a bandage over the upper part of the leg for 20 minutes. The test site was examined after 1, 2, 5, and 24 hours (modified from Ryan [12]). A positive test consisted of urticarial lesions under and around the pressure site, accompanied by a tender, deep seated, hard edema of the subcutaneous tissue.

Cold Urticaria (16 patients). The patients were questioned for evidence of onset of lesions after cooling of the skin by contact with cold water snow, ice, cold foods, or wind. Symptoms after rewarming were also

noted. Patients included in this group had one or more positive test results in the following group of three tests:

1. In all patients, an ice cube was applied to the skin of the upper arm for ten minutes, followed by rewarming. A positive test consisted of weal formation under and immediately around the contact place of the ice cube.
2. In patients with a suspected history of cold urticaria and a negative ice cube test, the lower part of the arm (with venous congestion) was exposed to water of 4° C, 12° C and 21° C for ten minutes. A positive test showed diffuse erythema and edema of the arm, with or without weal formation.
3. In patients with a suspected history and no reaction to the first two tests, the patient, dressed in light clothes, was exposed to a temperature of 4° C in a room for ten minutes. A positive test consisted of erythema and urticaria of the exposed skin.

Cholinergic Urticaria (10 patients). Patients with a history of symptoms after sweating caused by exertion, nervous excitement or exposure to a warm environment were studied for the presence of cholinergic urticaria. One or both of the following tests were positive.

1. The patient was exposed to a half-full bath at a temperature of 42° C for ten minutes, causing profuse sweating. Positive tests consisted of the appearance of multiple small weals with flare on the upper half of the body after 10 to 30 minutes.
2. The patient received an intracutaneous injection of 0.1 ml of carbachol 1/4.000 on the volar aspect of the lower arm.[13] A positive test consisted of the appearance of minute satellite weals around the injection site. The onset is within a few minutes after injection. This test is positive only in the more severe cases.

Angioneurotic Edema (6 patients). Angioneurotic edema, swelling of the subcutaneous tissues of eyelids, lips, earlobes or dorsae of the hands, occurred at one time or another in 55 % of the patients, especially in the groups with chronic idiopathic urticaria, pressure urticaria and cholinergic urticaria. There was a group of six patients however, that included four with angioneurotic edema exclusively and two others with only occasional weals elsewhere. Hereditary angio edema was ruled out by a negative family history and normal values for C1 esterase inhibitor and for C3 and C4. All test values for physical urticaria were negative

in these patients. They were slightly younger (age range 15 to 37 years, median 26 years) than the remaining group of patients. There were no other findings discriminating them from the other groups.

Idiopathic Chronic Urticaria (31 patients). In the remaining 31 patients all physical tests gave negative results, no clear cut cause could be detected.

Controls. This group consisted of 70 individuals. They had psoriasis, acne vulgaris, crural ulcers or venereal diseases. Patients with immunological and/or internal diseases were deleted from the series.

Age and Sex Distribution

In the patient group, there were 50 women and 42 men. The ages ranged from 7 to 80 years, with a mean age of 33.1 years and a median of 30 years. The age and sex distribution of the control group was comparable to that of the patient group. There were 39 women and 31 men. The ages ranged between 12 and 75 years, with a mean of 33.2 years and a median of 30 years.

Laboratory Measurements

Measurements of protease inhibitors and complement factors were performed with citrate plasma (1 ml 3,8 % sodium citrate plus 9 ml venous blood, centrifugated at 2.000 rpm for two hours at 4° C). Plasma was stored in aliquots of 0,5 ml in polyethylene tubes at minus 85–90° C. All measurements were done in polyethylene vessels or tubes to prevent activation of plasma factors by glass contact. The plasma was collected at one of the first visits or, if the patients had been using medications, after a period free from antihistamines and corticosteroids. Most patients had active lesions at the time of plasma collection.

Methods for determination of levels of plasma inhibitors for trypsin α-chymotrypsin, urinary kallikrein and C1 esterase have been described in detail in an earlier paper [14] (see chapter 4).

Trypsin, α-chymotrypsin, and C1 esterase inhibitor capacity were measured titrimetrically with synthetic esters as substrate: N-α-benzoyl-L-arginine ethyl ester (BAEe) was used for trypsin and N-acetyl-L-tyrosine

ethyl ester (ATEe) for chymotrypsin and C1 esterase respectively. Kallikrein inhibition levels were determined spectrophotometrically with N-α-tosyl-L-arginine methylester (TAMe) as substrate.

The amount of plasma used in each assay was chosen in such a way that 10 % to 60 % of the diluted enzyme preparation was inhibited (linear inhibition curves). The amounts of plasma used were: for trypsin 0.002 ml, for chymotrypsin 0.0004 ml, for urinary kallikrein 0.2 ml, and for C1 esterase 0.1 ml plasma. The assays were performed in duplicate with the diluted plasma (without enzyme) as a blank. Trypsin, chymotrypsin and kallikrein inhibitor levels are expressed as the amount (in milligrams) of a standard preparation of the enzyme inhibited by one milliliter of plasma (corrected for dilution with citrate solution).

Data obtained with different trypsin, chymotrypsin or kallikrein preparations can be compared with each other by computing the amount of enzyme (in milligram) inhibited by 1 ml of plasma with the activity of the enzyme preparation (results expressed as μMol substrate hydrolysis inhibited/minute/ml of serum).[6] Activity of trypsin (ex bovine pancreas) was 70.1 μMol H^- released from BAEe at pH 8 and 37° C per minute per milligram enzyme. Activity of α-chymotrypsin (ex bovine pancreas) was 225 μMol H^+ released from ATEe per minute per milligram enzyme Activity of human urinary kallikrein (isolated from urine of healthy men) was 2 μ Mol H^+ released from BAEe per minute per milligram (at pH 8, 37° C, 5 milligram BAEe per 3.5 milliliter saline). Cl esterase inhibition, expressed as μ Mol ATEe, inhibited by 1 milliliter of plasma of a standard amount of euglobulin preparation (activity 0.2 μ Mol ATEe/assay, in 3.2 milliliter saline, 2 milligram ATEe, pH 8, and 37° C.) Under this condition 0.1 milliliter of plasma gave about 50 % inhibition.

The concentration of α_1-antitrypsin, α_2-macroglobulin, and the complement factors C3 and C4 were measured with the Mancini technique with the aid of Partigen immunodiffusion plates.[14] Results are expressed as milligram protein in 100 ml of blood. The values are given as the mean plus or minus 1 SD (standard deviation). Statistical analysis for significance of the differences of the means was performed by Student's t test.

Skin Tests with Kallikrein

Studies performed by Juhlin and Michaelsson [2] indicate that many patients with idiopathic chronic urticaria show a substantially increased

skin reactivity to padutin, a kallikrein preparation from hog pancreas. They postulated that these patients had a kallikrein-inhibitor deficiency.

To verify this assumption, intracutaneously given tests were performed with a kallikrein preparation isolated from human urine. Urinary kallikrein is assumed to be a tissue kallikrein that is quite similar to the kallikrein found in human skin and sweat. The reactions were compared with those to padutin.

Urinary kallikrein was isolated from urine of healthy men (20 to 40 years old) by a batch procedure with diethylaminoethyl (DEAE) cellulose. The active esterase was absorbed to the cellulose at pH 7 and was eluted from it by decreasing the pH to 6 and increasing the molarity to 0.5 M sodium chloride. The crude kallikrein preparation was purified by means of ammonium sulphate and acetone precipitation and by means of column chromatography on Sephadex G-100 and DEAE cellulose (with a NaCl gradient). The kallikrein used had an activity of 2.00 μMol H^+ released from N-α-benzoyl-L-arginine ethyl ester (BAEe) per minute per milligram enzyme (at pH 8, 37° C, 5 mg BAEe/3.5 ml saline). The preparation had no urokinase activity as assayed with the method described by Boomgaard.[15]

Intracutaneous tests were performed with 0.1 ml of the urinary kallikrein solution in saline (pH 7) containing 1 mg/ml esterase. Padutin was injected intracutaneously in an amount of 2 kallikrein units (1 KU has an activity of 0.300 μMol H^+ released per minute under the same conditions as above). Tests with urinary kallikrein and padutin were done on the forearm of 16 patients with idiopathic urticaria, four patients with pressure urticaria, four with cold urticaria, two with cholinergic urticaria, one with urticaria factitia, and one with acquired angioneurotic edema. The controls were 19 patients with other skin diseases from the same sex and age group.

The reactions were determined after 20 minutes, and two, five, and 24 hours by measuring the diameters of the weals (edema, induration) and of the erythema.

RESULTS

In the group of patients with cold urticaria, changes were found in the protease inhibitor values (Table 2). They had a lowered capacity for trypsin inhibition ($p < 0.01$ by Student t test) and decreased plasma concentrations of α_1-antitrypsin ($p < 0.001$, Fig. 1).

TABLE 2. PROTEASE INHIBITORS IN PATIENTS WITH CHRONIC URTICARIA

patients	N	trypsin inhibition capacity	chymotrypsin inhibition capacity	kallikrein inhibition capacity	α_1-antitrypsin	α_2-macroglobulin
cold contact urticaria (acquired)	16	1.058±0.289* $p \leqq 0.01$	0.541±0.308* $p = 0.05$	0.126±0.031*	199±45* ($n = 15$) $p < 0.001$	280±83*
angioneurotic edema (acquired)	6	1.036±0.289 ($n = 12$) $p = 0.01$	0.332±0.160 $0.001 < p < 0.01$	0.109±0.057 $p = 0.02$	204±41 ($n = 12$) $0.001 < p < 0.01$	275±83
urticaria factitia	17	1.288±0.370	0.529±0.197	0.137±0.026 ($n = 16$)	287±114	247± 69
pressure urticaria	12	1.362±0.252	0.861±0.283	0.124±0.043 ($n = 11$)	310±113	219± 63
urticaria cholinergica	10	1.295±0.281	0.775±0.258	0.165±0.065	292± 50	247±104
idiopathic chronic urticaria	31	1.288±0.400	0.677±0.246	0.146±0.031	288± 80 ($n = 29$)	249±104 ($n = 29$)
controls	68	1.251±0.266	0.701±0.332	0.139±0.033	273± 72 ($n = 57$)	268± 83 ($n = 57$)

* ± plus or minus one standard deviation
< less than

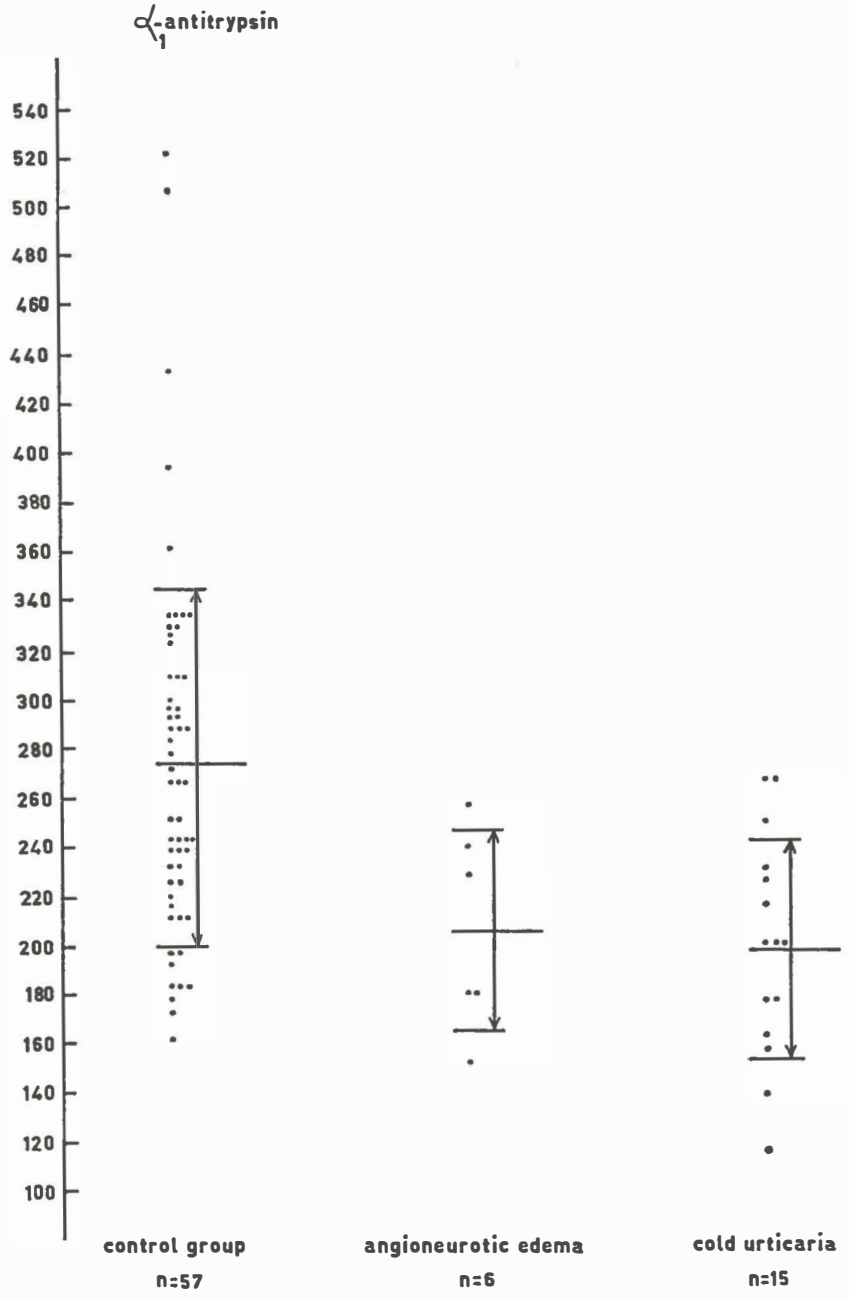

Fig. 1. Scattergram of α_1-antitrypsin levels (in mg/100 ml of plasma) in patients with angioneurotic edema, in patients with acquired cold urticaria and in controls. Arrows represent mean plus or minus one standard deviation.

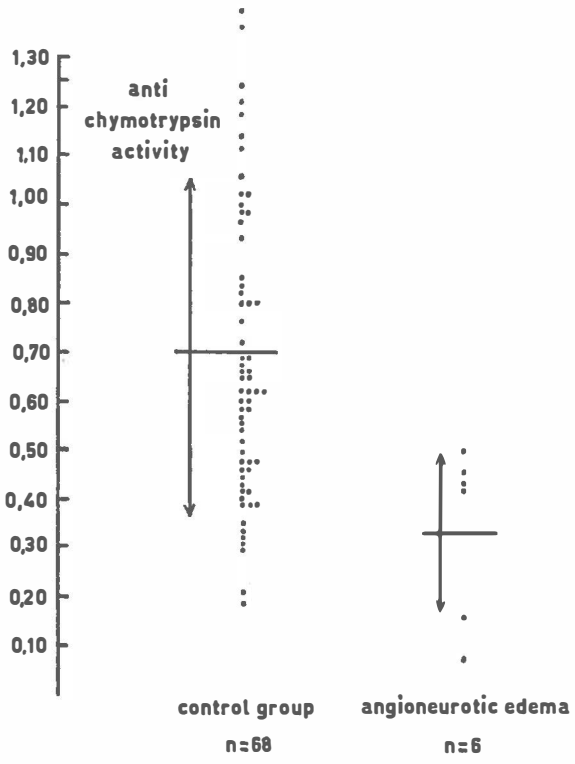

Fig. 2. Scattergram of antichymotrypsin activity (in mg chymotrypsin inhibited by one ml of plasma) in patients with angioneurotic edema and in controls. Arrows represent mean plus or minus one standard deviation.

The most remarkable changes were seen in the group with angioneurotic edema (Table 2), in which α_1-antitrypsin ($p = 0.001$ to 0.01) (Fig. 1) and chymotrypsin inhibition ($p = 0.001$ to 0.01) (Fig. 2) were decreased. Trypsin ($p = 0.01$) and kallikrein inhibition ($p = 0.02$) were less lowered compared to the controls. There were no significant changes in the protease inhibitor values of the other groups of urticaria patients.

The values for C1 esterase inhibition and the complement factors C3 and C4 were within normal range in all patients (Table 3). The plasma concentrations of protease inhibitors and complement factors were found to be independent of age.

In 28 patients, intracutaneous tests were done with urinary kallikrein. Eight out of 14 patients with idiopathic chronic urticaria showed sub-

TABLE 3. COMPLEMENT FACTORS IN CHRONIC URTICARIA PATIENTS

patients	N	C1 esterase inhibition	C3	C4
cold contact urticaria (acquired)	16	1.10±0.30*	73±14*	29.5± 9.9*
angioneurotic edema (acquired)	6	1.08±0.21	70±24	36.3±13.3
urticaria factitia	17	1.04±0.22	84±18	34.6±14.8
pressure urticaria	12	1.09±0.24	84±21	41.0±15.6
urticaria cholinergica	10	0.91±0.32	75±17	34.4±12.0
idiopathic chronic urticaria	31	0.98±0.34	85±13 n = 29	35.3±11.1 n = 29
controls	65	0.86±0.28	80±14 n = 57	32.5±10.7 n = 57

* ± plus or minus one standard deviation.

stantially edematous and painful infiltrates over areas larger than 1.000 sq mm. The reactions were most intense after five hours, which was also the case in Juhlin's patients. No abnormalities were found in the other types of patients (one with angioneurotic edema, two with urticaria factitia, four with pressure urticaria, five with cold urticaria and two with cholinergic urticaria) or in the controls. There was no correlation between increased skin reactivity to kallikrein and a decrease of protease inhibitor values (Table 4).

TABLE 4. CORRELATION OF SKIN REACTIVITY TO KALLIKREIN AND PLASMA LEVELS OF PROTEASE INHIBITORS

patients	N	C1 esterase inhibition	trypsin inhibition	chymo-trypsin inhibition	urinary kallikrein inhibition
idiopathic chronic urticaria patients with strong reactions to kallikrein*	8	0.95±0.20†	1.339±0.226†	0.664±0.135†	0.152±0.036†
controls	68	0.86±0.28	1.251±0.266	0.701 ±0.332	0.139±0.033

* induration > 1.000 sq mm five hours after injection.
† plus or minus one standard deviation.

Our results confirm those of Juhlin and Michaëlsson [2] with regard to an increased susceptibility to kallikrein in many patients with idiopathic chronic urticaria. However, our results do not support their hypothesis of the occurrence of a deficiency in protease inhibitors in these patients.

DISCUSSION

Several studies of large groups of patients with chronic urticaria indicate that the cause of the eruption is not found in 30 to 80 % of cases.[16,17] This probably reflects the insufficient state of our knowledge of the causal processes that can provoke urticarial responses.

Histamine, released from mast cells and basophils, undoubtedly plays a central role in urticaria, particularly in the evanescent type. Release may occur in type I hypersensitivity reactions and as a secondary effect of other mediators.

Histamine has a transient effect, and it is difficult to attribute urticarial lesions of longer duration to histamine release alone. Moreover, in these patients the lesions are often resistant to the effect of antihistamines.[16]

Attention has been directed therefore to other mediator systems, and some evidence has accumulated that these systems may be involved in the pathogenesis of chronic urticaria. These include the kallikrein-kinin system,[2,18–21] the complement system,[4,22] and the clotting-fibrinolysis system.[12,23] The prostaglandins also have to be considered.[24–26]

Potential elicitors of weal and flare type reactions in human skin are, next to histamine, the kinins (bradykinin, kallidin), the complement factors C3a, C5a and C2 peptide, the prostaglandins PGE_1 and PGE_2, and probably also slow-reacting substance of anaphylaxis.[4,22] Some of the probable release mechanisms are shown in Table 5. The various mediator systems are closely interrelated. They consist of cascades of enzyme-substrate interactions, held in balance by inactivators and inhibitors.

If an inhibitor is absent or deficient, an overflow of one or more mediators may occur.[27] This is the case in HEAE patients[3,4] whose plasma is deficient in C1 esterase inhibitor. Another example of congenital absence or deficiency of an inhibitor leading to disease is the α_1-antitrypsin deficiency occurring in patients with familial pulmonary emphysema and infantile liver cirrhosis.[29]

Acquired deficiencies in protease inhibitors were postulated by Juhlin and Michaëlsson [2] in patients with idiopathic chronic urticaria with negative tests for physical urticarias. Their hypothesis was based on the

TABLE 5. POTENTIAL MEDIATORS IN URTICARIA AND ANGIONEUROTIC EDEMA

substance	activity	important release mechanisms
histamine	vasopermeation, vasodilatation, and contraction of smooth muscles	type I hypersensitivity reactions immune complex diseases (type III) release by histamine liberators mastocytosis (non-specific stimulation)
slow-reacting substance of anaphylaxis	vasopermeation and contraction of smooth muscles	from mast cells from polymorphonuclear leukocytes
kinins (bradykinin, kallidin)	vasopermeation, vasodilatation, pain, and chemotaxis of leukocytes	activation of kallikrein system fibrinolysis, clotting system by proteases and lysosomal enzymes activation by collagen, glass, heat, ultraviolet, cold
anaphylatoxins (C3a, C5a), and C2 peptide	vasopermeation, contraction of smooth muscles, and chemotaxis (via histamine release)	activation of complement system, either immunologically or non-immunologically
prostaglandins PGE_1 and PGE_2	vasodilatation and vasopermeation	inflammatory processes leading to enhanced synthesis of prostaglandins

observation, that many of these patients were extremely sensitive to intracutaneously administered injections of bradykinin and kallikrein. Kallikrein is the enzyme that releases kinin from the inactive precursor, kininogen. Our study corroborates their observation of an increased sensitivity to kallikrein.

However, no correlation was found between increased skin sensitivity to kallikrein and decreased levels of protease inhibitors. On the other hand, we did find decreased levels of protease inhibitors in patients with acquired cold contact urticaria. Significant differences were observed in the level of α_1-antitrypsin and total antitrypsin activity in 16 patients with cold urticaria as compared to controls. In a study of Duck *et al.*,[1] substantially decreased values of antitrypsin and antichymotrypsin activities were observed in 12 patients with cold urticaria. In 29 of Duck's patients with idiopathic chronic urticaria, normal levels were observed. Some results obtained by Duck *et al.* suggest that the fall in inhibitor concentration correlates with the severity of the lesions provoked by cold exposure.

It is likely that in acquired cold urticaria, more than one causal factor is responsible for the symptoms. These patients form a heterogeneous group. Temperatures at which the symptoms are provoked vary from 0 to 22° C in different patients. In some cases passive transfer is possible, in others it is not.[28] Possibly patients with substantially decreased levels of α_1-antitrypsin (200 mg/100 ml or less) may also form a special subgroup. Preliminary results indicate that trypsin inhibitor levels, particularly in patients with low concentrations, remain relatively constant over a period of at least 12 months, irrespective of whether plasma was withdrawn during an active phase or in a symptom-free period.

Serum α_1-antitrypsin is inherited via a series of more than 11 different codominant protease inhibitor alleles, each of which is completely expressed.[29] Primary deficiencies of α_1-antitrypsin inhibitor levels (10 % to 15 % of the normal amount) are highly associated with early onset emphysema and infantile liver cirrhosis.[30]

Similarly, in chronic cold urticaria, an inhibitor deficiency may act as a predisposing factor for the development of lesions on cold exposure. The direct cause of the inflammatory response may be the activation of the kallikrein system by cold.[31] It cannot be excluded, that the decreased levels of antitrypsin activity are secondary and are due to binding of the inhibitor to other plasma factors. Protease inhibitor typing of α_1-antitrypsin in particular patients with cold urticaria will probably clarify this

point. These analyses are underway now, combined with longitudinal studies covering periods of over one or more years.

It is noteworthy that, in patients with familial cold urticaria normal levels of protease inhibitors were found,[14] but during cold exposure, chymotrypsin inhibition capacity was observed to show a definite fall while α_1-antitrypsin concentrations increased.

The most prominent changes were observed in patients with acquired angioneurotic edema. These individuals differ fromt those with the hereditary form of the disease in several aspects. Patients with HEAE have low levels of C1 esterase inhibitor and decreased levels of C4 and C2. In one form of acquired angioneurotic edema, in patients with lymphosarcoma, C4 has also been found to be low.[32] In our patients, the levels of C1 esterase inhibitor and of C4 were normal. However, decreased levels of α_1-antitrypsin and chymotrypsin inhibitor and a less definite decrease of anti-kallikrein capacity levels were observed. What the eliciting factor is in these patients is not yet clear.

There is evidence of decreased levels of protease inhibitors in patients with acquired cold urticaria and acquired angioneurotic edema. These changes suggest a possible role in the pathogenesis of the disease. Further studies, especially of experimental cold exposure and longitudinal studies, will have to be carried out to elucidate the exact role of the abnormalities found.

SUMMARY

The hypothesis that deficiencies of plasma protease inhibitors might play a role in the pathogenesis of chronic urticaria was evaluated. Plasma levels were measured in patients with urticaria and a matched control group for α_1-antitrypsin, α_2-macroglobulin, total trypsin-inhibiting capacity, kallikrein-inhibiting capacity, and the complement factors C1 esterase inhibitor, C3 and C4.

A total of 92 patients with chronic urticaria of more than three month's duration was studied. Patients with acquired cold urticaria had significantly decreased levels of α_1-antitrypsin and total antitrypsin activity. In patients with acquired angioneurotic edema, α_1-antitrypsin levels and antichymotrypsin activities were lowered, with less significant decreases in antitrypsin and antikallikrein activities. Levels of C1 esterase inhibitor, C3, and C4 were normal in all groups. There was no correlation between the increased sensitivity to intracutaneously administered kallikrein injection and deficiencies of protease inhibitors.

REFERENCES

1. Duck HJ, Barth J, Wagner J: Die antiproteolytische Kapazität des Serums bei verschiedenen Urtikariaformen. *Dermatol Monatsschr* 157: 491–499, 1971.
2. Juhlin L, Michaëlsson G: Cutaneous reaction to kallikrein, bradykinin and histamine in healthy subjects and in patients with urticaria. *Acta Derm Venereol* 49: 26–36, 1969.
3. Hadjiyannaki K, Lachmann PJ: Hereditary angio-edema: A review with particular reference to pathogenesis and treatment. *Clin Allergy* 1:221–233, 1971.
4. Valentine MD, Sheffer AL, Austen KF: Urticaria and angioedema, in Samter M: *Immunological Diseases*, 2. Boston, Little, Brown & Co, 1971, pp 909–919.
5. Bieth J, Métais P, Warter J: Détermination de la capacité d'inhibition trypsique et chymotrypsique du sérum humain normal. *Enzym Biol Clin* 10: 243–257, 1969.
6. Dietz AA, Hodges LK, Rubinstein HM et al: Estimation of the antitrypsin activity of serum. *Clin Chem* 13: 242–254, 1967.
7. Fritz H, Trautschold I, Haendle H, et al: Chemistry and biochemistry of proteinase inhibitors from mammalian tissues. *Ann NY Acad Sci* 146: 400–413, 1968.
8. Harpel PC: Studies on human plasma α_2-macroglobulin-enzyme interactions. *J Exp Med* 138: 508–521, 1973.
9. McConnell DJ: Inhibitors of kallikrein in human plasma. *J Clin Invest* 51: 1611–1623, 1972.
10. Ratnoff OD, Pensky J, Ogston D, et al: The inhibition of plasmin, plasma kallikrein, plasma permeability factor and C1r subcomponent of the first component of complement by serum Cl esterase inhibitor. *J Exp Med* 129: 315–331, 1969.
11. Grolnick M: An investigative and clinical evaluation of dermographism. *Ann Allergy* 28: 395–404, 1970.
12. Ryan TJ, Shim-Young N, Turk JL: Delayed pressure urticaria. *Br J Dermatol* 80: 485–490, 1968.
13. Illig L: Physical urticaria: Its diagnosis and treatment. *Curr Probl Dermatol* 5: 79–116, 1973.
14. Doeglas HMG, Bleumink E: Familial cold urticaria: Clinical findings. *Arch Dermatol* 110: 382–388, 1974.
15. Boomgaard J: Urokinase, een onderzoek naar de uitscheiding en functie, *Thesis*. Amsterdam, 1965.
16. Champion RH, Roberts SOB, Carpenter RG, et al: Urticaria and angioedema: A review of 554 patients. *Br J Dermatol* 81: 588–597, 1969.
17. Green GR, Koelsche GA, Kierland RH: Etiology and pathogenesis of chronic urticaria. *Ann Allergy* 23: 30–36, 1965.
18. Aoyama H: Chemical mediation of urticaria factitia. *Jap J Dermatol* 79: 379–384, 1969.
19. De Laus FV, Winkelmann RK: Kinins in cold urticaria. *Arch Dermatol* 98: 67–74: 1968.
20. Lecomte J, et al: Formation de kinines plasmatiques dans les lésions d'urticaire 'à frigore'. *Rev Fr Allergol* 13: 3–9, 1973.
21. Winkelmann RK: Chronic urticaria. *Proc Mayo Clinics* 69: 361–370, 1968.
22. Mathews KP: A current view of urticaria. *Med Clin North Am* 58: 185–205, 1974.
23. Ryan TJ, Nishioka K, Dawber RPR: Epithelial-endothelial interaction in the control of inflammation through fibrinolysis. *Br J Dermatol* 84: 501–515, 1971.
24. Jörgensen HP, Søndergaard J: Vascular responses to prostaglandin E_1. *Acta Derm Venereol* 53: 203–206, 1973.
25. Juhlin L, Michaëlsson G: Cutaneous vascular reactions to prostaglandins in healthy subjects and in patients with urticaria. *Acta Derm Venereol* 49: 251–261, 1969.

26. Moncada S, Ferreira SH, Vane JR: Prostaglandins, aspirin-like drugs and the edema of inflammation. *Nature* 246: 217–219, 1973.
27. Movat HZ: Chemical mediators of the vascular phenomena of the acute inflammatory reaction and of immediate hypersensitivity. *Med Clin North Am* 56: 541–556, 1972.
28. Houser DD, Arbesman CE, Ito K, et al: Cold urticaria: Immunologic studies. *Am J Med* 49: 23–33, 1970.
29. Talamo RC, Langley CE, Levine BW, et al: Genetic vs quantitative analysis of serum alpha$_1$-antitrypsin. *N Engl J Med* 287: 1067–1069, 1972.
30. Talamo RC: The α_1-antitrypsin in man. *J Allergy* 48: 240–249, 1971.
31. Armstrong D: Actions on the human, cold-activated plasma kinin forming system of pre-heating at 56° and 60°. *Pharmacol Res Commun* 1: 30–35, 1969.
32. Caldwell JR, Ruddy S, Schur PH, et al: Acquired $C\bar{1}$ inhibitor deficiency in lymphosarcoma. *Clin Immunol Immunopathol* 1: 39–52, 1972.

CHAPTER 4

FAMILIAL COLD URTICARIA. CLINICAL FINDINGS*

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INTRODUCTION

Familial cold urticaria (FCU) is an autosomal dominant hereditary disease, first described by Kile and Rusk in 1940.[1] Since then five more families have been reported from the United States [2-6] and one from France [7] (Table 1). In all cases, the onset is within the first weeks of life. The symptoms occur on generalized exposure to cold air, especially in combination with damp and windy weather. After an interval of one half to several hours, a rash develops, consisting of urticarial papules and erythematous macules on the exposed skin, that causes a burning sensation. After severe exposure, the rash may spread to the covered skin and this may be accompanied by fever, chills, joint pains, stiffness and swelling of the hands and feet. These manifestations last up to several days, depending on the degree of exposure. Patients with severe cases are incapacitated by the disease.

Familial cold urticaria has to be differentiated from acquired contact cold urticaria which, apart from occurring sporadically, has features of an immediate type allergic reaction. In acquired contact cold urticaria, the reaction can usually be elicited within minutes after contact of the skin with an ice cube. The lesions are more typically urticarial, mast cell degranulation has been demonstrated[8] and the passive transfer test is positive in 50 % of cases.[9] All these features are absent in FCU.

Another distinguishing symptom is the presence of a leukocytosis preceding and accompanying the cold reaction in FCU.[4-6] Also in contrast to other urticarial processes, the dermis in FCU has been found to contain an intense polymorphonuclear leukocytic infiltrate.[5]

Little is known about the pathogenesis of familial cold urticaria. Tindall *et al.*[5] suggested that humoral factors, generated by cooling of the skin, initiate the reactions that lead to visible skin responses. The humoral factor is in all probability not histamine, but from histological findings in

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patients with active lesions of FCU, it may be deduced that vasoactive components are released. Moreover, leukocytosis is a constant feature of the generalized reaction, suggesting the release of a chemotactic factor.

One of the possible explanations might be, that patients with FCU have a labile blood enzyme system, either due to the absence of certain inhibitors for proteolytic enzymes, or due to the presence of (pro) enzymes, which are easily activated by cold exposure.

There are several examples of deficiencies in blood protease inhibitors. A genetically determined deficiency of α_1-antitrypsin has been shown to be associated with pulmonary emphysema among infants. Hereditary angioneurotic edema is a second example. In these patients a low C1-esterase inhibitor capacity is found.

Therefore, we undertook a systematic study of the plasma enzymes and their inhibitors in the kallikrein, clotting and complement systems. We started with measurements of the capacities of plasma to inhibit trypsin, chymotrypsin, C1-esterase, urinary kallikrein and of complement factors.

The purpose of the present paper is to describe the clinical features of FCU more closely, to report results of experimental cold exposure in two patients and to present data on the levels of enzyme inhibitors and complement factors in the group of patients during remission and during experimental cold exposure.

Separate from this study, a genetic investigation has been made. The results of linkage studies with various genetic markers have been published elsewhere[10] (see chapter 5). A preliminary note about this family was published previously.[11]

METHODS

Family data were collected from local registry offices. All members were visited at home by the author. Histories were taken on standard questionnaire sheets. At each visit, the family history was cross checked with special attention given to details about deceased members.

At the time of the visit, blood was drawn for chemical and linkage studies (5 ml clotted blood, 5 ml in ethyl diamine tetra acetic acid solution, and 5 ml in acid citrate dextran solution).

The tubes were transferred to the laboratory within 12 hours and frozen at $-70\,^{\circ}C$. All white blood cell counts were made in duplicate with a Coulter counter.

Levels of serum inhibitors for trypsin, chymotrypsin, urinary kallikrein

TABLE 1. REVIEW OF PUBLISHED FAMILIES WITH FAMILIAL COLD URTICARIA

author, yr	Kile, 1940	Urbach, 1941	Witherspoon, 1948	Rodin, 1951	Tindall, 1959	Derbes, 1972	Castelain, 1971	Doeglas, 1974
country	US	US	US	US	US	US	France	Netherlands
age at onset	"all her life"	birth	birth	birth	birth	birth	birth	0–5 years
interval, hr	½	1–2	1–2	½	½–5	1–2	½	½–3
maximum duration, hr	48	48	6	½ hr after rewarming	24	72	2	48
weather	damp cold	damp cold	cold, windy	cold	cold, windy	cold, wind, handling ice	cold water	damp cold
worst at age	–	–	–	16–17 yrs	childhood	–	–	10–20 yrs
skin lesions	erythema, urticarial	erythema, urticarial	urticarial	urticarial	maculo-papular purpuric	urticarial	urticarial, erythematous plaques	urticarial, erythematous plaques, petechiae
localization	exposed skin	face, extremities	extremities, trunk	face, extremities	extremities, trunk	face, extremities, trunk	neck	extremities, trunk
subjective symptoms	burning	pain	burning	non-pruritic	burning	–	burning	itching, burning
joint symptoms	stiffness	swelling	pain, swelling	pain	pain	pain, swelling	–	pain, swelling
constitutional symptoms	fever, chills, headache	fever, headache, sleepiness	fever, chills	headache, perspiration, nausea	fever, chills, headache, nausea, thirst	fever	–	fever, chills, drowsiness
leukocytosis	–	–	–	23,800/cu mm	34,000/cu mm	–	10,400/cu mm	14,700/cu mm
experimental induction	arm bath 10 C, 25 min	cold room −12 C, 25 min	–	–	cold room 2 C, 6 hrs	–	–	cold room 6 C, 2 hrs
pedigree affected/total	23/42	17/28	24/44	20/35	18/38	36/74	3/6	11/19

and C 1-esterase were measured in citrate plasma according to methods that will be described in detail elsewhere (unpublished data). In short, the methods are as follows.

Venous blood was obtained in plastic (polythene) tubes (1 ml of 3.8 % sodium citrate plus 9 ml of blood), centrifugated at 2,000 g, and the plasma stored in aliquots of 0.5 ml in polythene tubes at −85 °C. Trypsin (EC 3. 4. 4. 4.), α-chymotrypsin (EC 3. 4. 4. 5.) and the C1-esterase inhibiting capacity of plasma were measured titrimetrically with the aid of a pH-stat with the use of synthetic esters as substrate. The measurements were done by constant pH titration–titrant consumed vs time–with the aid of a titrator coupled to an autoburette and a titrigraph.

As substrates, we used N-α-benzoyl-L-arginine-ethylester-hydrochloride (BAEe) for trypsin activity and N-acetyl-L-tyrosine-ethylester monohydrate (ATEe) for chymotrypsin and C1-esterase activity. The standard consisted of substrate plus enzyme; the test solution contained substrate, enzyme and diluted plasma (the blanks of plasma alone showed no activity). The difference between the standard and the test solution gives the amount of enzyme inhibited by 1 ml of plasma.

Conditions of Trypsin-Inhibiting Capacity.–Standard.–Trypsin, 5 μg/3.5 ml of saline (+0.01M $CaCl_2$); 5 mg BAEe; pH 8; and 37 C.

Titration was done with 0.01M NaOH under N_2.

Activity was measured in polyethylene vessels containing 3.5 ml of the solution, which was constantly stirred.

Activity of trypsin (ex bovine pancreas) under these conditions: 70.1 μmol BAEe split/min/mg of enzyme.

Test.–Trypsin (as previously mentioned); 0.1 ml in a diluted plasma in a 1 : 50 ratio (= 0.002 ml); BAEe (as previously described) in total 3.5 ml of saline.

The solutions were preincubated (without substrate) for 30 minutes at 37 C in order to obtain complete inhibition. The inhibition curve (equals activity versus plasma amount) is linear in this concentration range. All determinations were done in duplicate.

Conditions of Chymotrypsin-Inhibiting Capacity.– Standard.–Chymotrypsin, 1 μg/3.5 ml of saline; 2 mg ATEe; pH 7.4; 37 C with titration as previously mentioned.

Activity of α-chymotrypsin (ex bovine pancreas): 225/μmol ATEe split/min/mg of enzyme.

Test.–Chymotrypsin (as previously mentioned); 0.02 ml in a diluted plasma 1 : 50 ratio (0.0004 ml). ATEe as previously described. No preincubation necessary.

Conditions of C1-Esterase Inhibition.–Standard.–Euglobulin, 0.1 ml in saline (prepared from pooled human serum as described by Donaldson (1966).[12] Activity of the euglobulin preparation 0.150 μmol H^+ released/min/0.1 ml; 2 mg ATEe in total of 3.5 ml saline; pH 8; 37 C.

Test.–Euglobulin (as previously described); 0.1 ml undiluted plasma; ATEe (as previously described).

Kallikrein Inhibition.–Kallikrein activity was measured spectrophotometrically after a modification of a method described in detail by Colman *et al.* (1969).[13]

Standard.–Soybean trypsin inhibitor, 0.1 ml (SBTI, 4 mg/ml; one mg of inhibitor inhibits 1.5 mg of trypsin); 0.1 ml urinary kallikrein; 0.5 ml phosphate buffer, pH 7.6. The mixture was incubated for 15 minutes at 37 C after which 1.5 ml N-α-tosyl-L-arginine-methylester hydrochloride (TAMe, 7 mg/ml) was added. After 30 minutes, the reaction was stopped with 0.5 ml trichloroacetic acid.

Test.–SBTI, 0.1 ml; 0.2 ml plasma (undiluted); 0.3 ml phosphate buffer; 0.1 ml urinary kallikrein.

Serum Blank.–SBTI, 0.1 ml; 0.2 ml plasma, and 0.4 ml phosphate buffer.

The methanol formed during the reaction is oxydized by $KMnO_4$ and measured with chromotropic acid spectrophotometrically (at 580 nm). The amount of methanol released can be read from a standard curve.

Urinary kallikrein (EC 3. 4. 4. 21) was isolated from pooled urine of healthy men (age 20 to 40 years) and was purified by means of precipitation with ammonium sulfate and acetone and by chromatography on Sephadex G 100 and DEAE-cellulose. A standard preparation of lyophilized urinary kallikrein with an activity of 2 μmol BAEe split/min/mg of enzyme (pH 8, 37 C, 5 mg BAEe/3.5 ml of saline) was used throughout the study. This preparation had no urokinase activity.

The concentrations of α_1-antitrypsin, α_2-macroglobulin and the complement factors C3 and C4 were determined with the Mancini technique with the aid of Partigen immunodiffusion plates. The plates contained 12 wells, three wells of which were filled with 5 μl, with the use of a Hamilton syringe, of three dilutions of a standard serum or of a protein standard solution (standard human serum batch 972 or 570 AK, protein standard serum batch 572). The other wells were filled with 5 μl of diluted sera or plasma (for C3, C4, and α_2-macroglobulin 1 : 2 and for α_1-antitrypsin determination 1 : 10). The plates were incubated for 48 hours at 20 C, then washed with several changes of saline to remove unprecipitated

protein after which the precipitation rings were made visible with Coomassie brilliant blue.

The squares of the diameters of the rings show a linear relationship with protein concentration. The concentration of the particular protein in the sample can be read directly from the standard curve of the reference serum.

THE FAMILY

The family lives in a rural area in the northeast of the Netherlands. Several members are farmers.

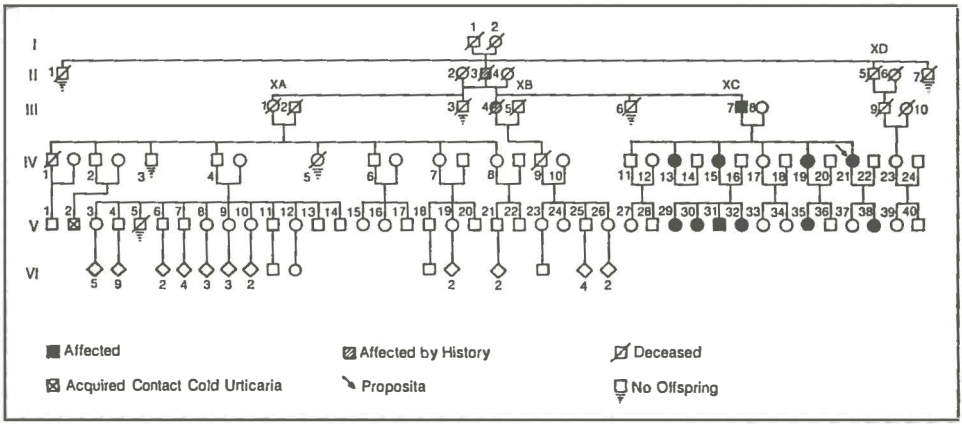
A genealogical study was made over six generations, including 108 family members (Fig. 1).

All cases of FCU descend from patient II-3, whose case is the first recorded in the family's history. This patient was married twice. There were 65 descendants from his first marriage (branch XA), all of whom were free of symptoms. There was one patient (V-2) who had symptoms of acquired contact cold urticaria by history. On examination an ice cube test was negative.

Of the three children of his second marriage (branch XB), two were affected. His oldest daughter (III-4) died at the age of 54; she had severe symptoms during her entire life. Her 12 descendants were symptom-free.

The youngest son (III-7) is our oldest living patient. In this branch of the family (XC), there were 11 cases among 19 members in three genera-

Fig. 1. Pedigree of Dutch family with FCU, updated through 1973.



tions. The FCU trait is transmitted only by affected members, to approximately half of their children and no generations are skipped.

There is no influence of sex, with the predominance of female patients (9 : 2) being a result of the preponderance of female children in the family. On the basis of these facts, autosomal dominant inheritance can be accepted.

Among the members of branch XC the symptoms and the genetic nature of FCU are well known. The patients learn to live with their complaints, rarely seeking medical help.

The proposita (IV-21) visited the Skin clinic when her youngest daughter began to have serious complaints.

All 11 patients were examined. The symptoms are shown in Table 2.

TABLE 2. CLINICAL FINDINGS IN 11

patient	III-7	IV-13	IV-15	IV-19	IV-21
sex/age, yr	M/79	F/52	F/50	F/40	F/37
age at onset, yrs	4–5	4	4–5	4–5	1
occupation	butcher	housewife	farmer	shopkeeper	farmer
interval maximum duration, hr	hours 16	hours 24	hours 48	hours 24	hours 72
season	not in summer	all	not in summer	winter	all
worst at age, yr	15–40	10–30	6–15	25	14–now
skin lesions	?	erythematous macules	bluish red macules	erythematous plaques	erythematous plaques, petechiae
localization	extremities	extremities	extremities	extremities	extremities
itching	–	–	+	–	+
burning	–	+	+	–	+
edema	+	+	+	+	+
joint pains, stiffness	+	+	+	+	+
constitutional symptoms	fever, chills	fever, chills	fever, tired, sleepy	fever, chills	fever
therapy	bed, stop work	bed, oven	bed, electric blanket	– hot water	bed, hot water
degree of involvement	severe	severe	moderate	slight	severe

* +, present; –, absent.

REPORT OF CASES

CASE 1(IV-21, Proposita).–A 37-year old woman lives in a farmhouse without central heating. Symptoms occur in all seasons, both indoors and outdoors, especially in damp and windy weather. Skin lesions usually appear one to three hours after exposure and may last from a few hours to several days. She was seen during an attack precipitated by bicycling for 30 minutes against the wind, when the temperature was 3 C. The skin changes began after three hours. She was seen six hours after exposure. Lesions were present on the dorsae of the hands and wrists and on the frontal and medial sides of the legs. They consisted of bright red to bluish-red elevated, discrete and confluent plaques with vague outlines and a diameter of 0.5 to 5 cm. The smaller lesions were surrounded by a pale halo. Some of the larger lesions showed central petechiae. The skin

CASES OF FAMILIAL COLD URTICARIA

V-29	V-30	V-31	V-32	V-35	V-38
F/24	F/21	M/18	F/12	F/15	F/6
I	1	5	6	4	birth
housewife	schoolteacher	student	student	student	student
hours	15 min	hours	hours	hours	hours
24	24	3	3	48	48
all	all	winter	winter	all	not in summer
21	now	9–11	?	?	?
erythematous macules	bluish red plaques	erythematous macules	erythematous macules	bluish red macules	erythematous plaques, urticaria
extremities, trunk	extremities, trunk	extremities, trunk	legs	extremities, trunk	extremities, trunk
+	+	+	–	+	+
+	–	–	+	–	+
+	+	+	+	+	+
+	+	+	+	+	+
chills	chills, drowsy, thirst	fever	chills	–	fever
–	bed,	bed	bed,	–	bed,
hot water	hot water, rubbing		hot water	electric blanket	hot water
slight	severe	slight	slight	moderate	severe

was hot to the touch. Subjectively, there was a burning and glowing sensation. The total white blood cell count (WBC) at that moment was 8,600/cu mm, with 77 % polymorphonuclear neutrophils, 1 % band forms, 5 % eosinophils, 1 % monocytes and 16 % lymphocytes. Antihistamines did not prevent her attacks, but seemed to mitigate them. Laboratory findings during a remission were normal. Normal values were found for serum cryoglobulins, serum cold agglutinins, serum electrophoresis, alkaline phosphatase, creatinine, transaminases, antistreptolysin titer, Rose test, VDRL test and Wassermann test, antinuclear fluorescence test, a complete blood cell count and urinalysis.

CASE 2 (V-30).–A 20 year old woman school teacher had had symptoms since the age of 4, especially in damp weather. Symptoms occurred in all seasons. Even a draft from an open window might cause small macules on the arms within 15 minutes. After more severe exposure, larger bluish-red macules appear on the extremities with swelling and pain in fingers, toes and heels. These were accompanied by chills and intense drowsiness, forcing her to spend much of her free time in bed. By avoiding exposure as much as possible, she managed to continue her work in wintertime.

The same battery of laboratory tests as in patient IV-21, was performed during a remission, with normal results.

CASE 3 (V-38).–The 6 year old daughter of proposita had symptoms since birth. When in her baby carriage, she developed swollen hands. In fall, winter and spring, especially in damp weather, she showed skin lesions about three hours after cold exposure. These were accompanied by severe itching and tenderness, making her cry. She was examined six hours after a bicycle ride in the cold. She had numerous elevated erythematous macules with a diameter of 0.5 to 3.0 cm surrounded by a large white halo, sometimes confluent to larger plaques with central clearing. They were localized on the exposed parts of the legs and on the buttocks.

CLINICAL CHARACTERISTICS

The clinical findings in our patients are summarized in Table 2 and are in good agreement with earlier reports.[1-7] Our cases show a range of symptoms from slight (V-31, V-35) to incapacitating (IV-21, V-30).

The average age at onset in our pedigree is 4 to 5 years, which is considerably higher than in other series. This may be a result of the mild Dutch climate, which might make symptoms go unnoticed in younger children. In the youngest patient, V-38, who was closely watched, symp-

toms were observed shortly after birth. In five of 11 patients, the symptoms occurred in all seasons, in three only in winter.

In seven patients dampness and in four wind were contributing factors. Wind and dampness probably cause a greater penetration of the cold and enhance generalized cooling. The effect of localized cooling is not clear in FCU. Other authors [1,6,7] report localized reactions after contact with ice and water. Our patients avoid cold water because of pain, but they do not develop lesions on incidental contacts.

The skin lesions in patients IV-21, V-30 and V-38 were observed on several occasions. They ranged from pinpoint bright red papular lesions to plateaulike elevated urticarial papules with a white halo and elevated bright red macules and plaques up to 15 cm in diameter. In all patients, these lesions were localized on the exposed skin of the extremities. In five patients, the upper extremities and gluteal areas were involved occasionally.

We agree with Tindall *et al.*[5] that the name familial cold urticaria is not justified by the variable nature of the skin lesions. that show similarity to those seen in a drug-induced rash. Nevertheless, earlier descriptions and our own observations suggest that urticaria is part of the clinical picture. In this connection, it is of interest that antihistamines do not prevent the symptoms of FCU but seem to mitigate them.[3] This has also been our experience with the three patients who received them.

Burning or glowing sensations of the skin lesions were present in six patients; seven complained of itching and four patients had both symptoms. All patients had swelling ('cushions') of the dorsa of the fingers and hands, pretibially, or on the lateral sides of the feet and on the heels. Painful swelling of the joints of hands and fingers with stiffness was also present in all patients. Chills and fever were observed in ten patients, and 8 had to rest in bed in order to recover. As far as the course of the disease is concerned, most of our patients noticed that their symptoms were most severe between the ages of 10 and 20 and improved thereafter with age. This is also suggested by the observations of others.[4,5]

EXPERIMENTAL AND LABORATORY FINDINGS

Histopathologic Findings.–A biopsy specimen of a macular lesion, with a duration of three hours, was taken from patient IV-21 at the height of an attack, after bicycling in the cold. It shows edema of the dermis and an infiltrate consisting mainly of polymorphonuclear leukocytes, espe-

TABLE 3. LEVELS OF INHIBITORS IN PLASMA OF MEMBERS OF FAMILY WITH FCU

persons tested	N	trypsin inhibition[a]	chymotrypsin inhibition[b]	kallikrein inhibition[c]	α_1-anti-trypsin[d]	α_2-macro-globulin[d]
all family members	19	1.071±0.229	0.677±0.258	0.146±0.039	251±23	304±105
patient group	11	1.043±0.229	0.750±0.209	0.151±0.033	254±75	320±78
healthy persons	8	1.080±0.200	0.566±0.271	0.135±0.035	247±28	284±139
controls	68	1.251±0.266	0.701±0.332	0.139±0.031	273±72 (n = 57)	268±83 (n = 57)

[a] expressed as milligrams of trypsin (activity 70.1 μmol BAEe split/minute/mg at pH 8 and 37 C; 5 mg BAEe/3.5 ml) inhibited by 1 ml of plasma.

[b] expressed as milligrams of chymotrypsin (activity 225 μmol ATEe split/minute/mg at pH 7.4 and 37 C; 2 mg ATEe/3.5 ml) inhibited by 1 ml of plasma.

[c] expressed as milligrams of human urinary kallikrein (activity 2 μmol TAMe split/minute/mg at pH 8.0 and 37 C; 5 mg TAMe/3.5 ml) inhibited by 1 ml of plasma.

[d] milligrams of α_1-antitrypsin (α_2-macroglobulin) present in 100 ml of plasma.

cially eosinophils around dilated capillaries and throughout the dermis. Some arterioles in the subcutis show infiltration of the vascular wall with mostly lymphocytes and some eosinophils.

The biopsy specimen of patient V-30 was taken from a subsiding lesion after cold room exposure. It shows more extensive changes with lymphocytic infiltrates perivascularly in the dermis, which increase toward and into the subcutis. At the border of the dermis and subcutis, there is swelling of endothelium and infiltration of vascular walls. A thrombotic arteriole is seen in this area. There are no signs of nuclear dust in either biopsy specimen.

TABLE 4. COMPLEMENT FACTORS IN PLASMA OF MEMBERS OF FAMILY WITH FCU

persons tested	N[a]	C1-esterase inhibition	C3[b]	C4[b]
all family members	19	0.82±0.19	81±12	27.5± 5.7
patient group	11	0.80±0.18	78±14	26.8± 4.6
healthy persons	8	0.93±0.24	83± 9	28.4± 7.3
controls	65	0.86±0.28	80±14 (n = 57)	32.5±10.7 (n = 57)

[a] assay: 0.1 ml plasma, 0.1 ml euglobulin solution (activity 0.200 μmol ATEe/min at pH 8.0 and 37 C), 3.2 ml saline (0.9 % NaCl) 2 mg ATEe, pH 8 and 37 C. Blank: the same but without plasma. Inhibition expressed as μmol ATEe inhibited per milliliter of plasma.

[b] milligrams of C3 (C4) present in 100 ml of plasma.

Plasma Protease Inhibitors and Complement Factors.–The plasma of 11 patients in remission was compared with that of the healthy members of the family and of 68 normal controls. There were no important differences in the levels of inhibitors for human urinary kallikrein, trypsin, chymotrypsin and C1 esterase (Table 3 and 4). The α_2-macroglobulin, α_1-antitrypsin and the complement factor C3 and C4 levels were within the normal range. A study of enzyme inhibitors in various other forms of chronic urticaria will be published elsewhere (see chapter 3).

EXPERIMENTAL INDUCTION OF SKIN LESIONS

Dressed in light clothes, patient IV-21, a woman of 37 years, stayed in a cold room at 6 C for two hours, and patient V-30, a woman of 19 years, stayed for five hours. Longer exposure was not tolerated because of a feeling of intense cold. Base line and hourly WBC and differential cell counts were made. Thrombocyte counts were made before and after exposure. Plasma and serum were obtained before, during and after exposure.

Both patients developed bluish-red macules on wrists and legs after two hours of exposure. They showed swelling of the dorsa of the hands and toes and had difficulty in closing their hands.

Fig. 2. Clinical response and rise in WBC count after cold-room exposure.

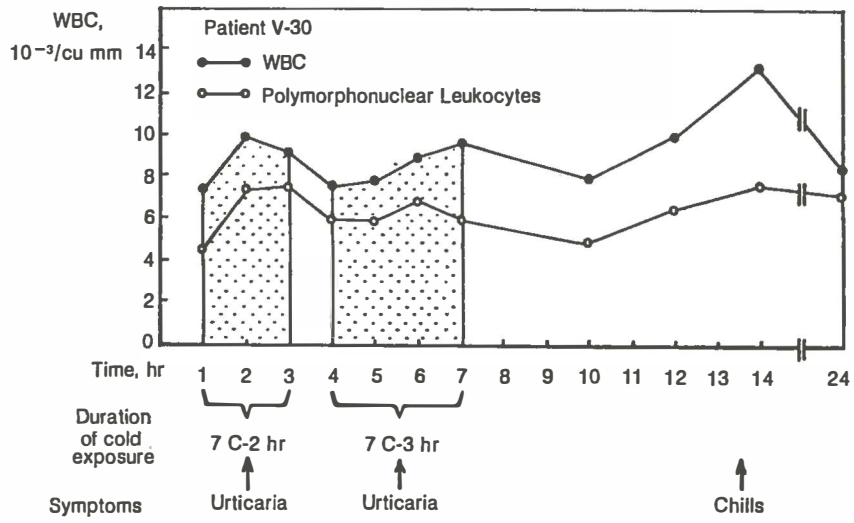


TABLE 5. LEVELS OF COMPLEMENT FACTORS AND PLASMA INHIBITORS DURING COLD EXPOSURE

time (hr)	C3[a]	C4[a]	α_1-anti-trypsin[a]	α_2-macro-globulin[a]	C1-ester inhibit[b]	trypsin inhibit[b]	chymotr inhibit[b]	urinary kallikr inhibit
0 (before exper)	75	28.0	255	286	1.066	1.031	1.218	0.098
7 (after exper)	75	26.6	266	263	1.233	1.702	0.381	0.098
24 (next day)	80	34.6	255	275	1.233	2.050[c]	0.702	0.074

[a] expressed as mg protein/100 ml serum.
[b] see footnotes to Tables 3 and 4.
[c] higher than the mean of control sera values +2 SD (SD 1.783).

During and after exposure, the WBC counts rose from 5,100 to 8,600/cu mm in patient IV-21 and from 6,700 to 13,400/cu mm in patient V-30 (Fig. 2), which was due to a rise in polymorphonuclear leukocytes. The thrombocyte counts remained unchanged. In Table 5, the data of the plasma components measured during cold exposure in patient V-30 have been collected.

A sharp decrease was observed in the chymotrypsin-inhibiting capacity, which fell from 1.218 mg/ml before exposure to 0.381 after seven hours. The next day it was still lowered compared with the initial value at 1.218. The mean of 68 controls was 0.701 ± 0.332.

A remarkable observation is, that the trypsin-inhibiting capacity increased from 1.031 to 2.050 mg/ml, whereas the α_1-antitrypsin concentration remained constant.

DISCUSSION

The full syndrome of FCU has been reproduced under rather extreme experimental conditions.[2,5] Under such conditions Tindall *et al.*[5] noted a rise in WBC count up to 34,000/cu mm preceding and accompanying the skin reaction. Considering the relatively mild exposures of our patients, our findings are not in disagreement. However, during a severe naturally occurring attack of patient IV-21, the WBC count was not excessively elevated.

The skin biopsy specimens of our patients showed less intense polymorphonuclear infiltrates than have been described.[5] In one patient, the changes suggested a vasculitis. The petechiae may be due to changes in

the vascular wall such as seen in patient V-30. The histopathologic findings of FCU may be more complex than has been suspected.

Familial cold urticaria has also been described as part of a syndrome combined with amyloidosis and deafness.[14-16] The family of the patient observed by McKusick and Goodman[15] was studied by Shepard and renal failure, probably due to amyloidosis was found to coincide with the urticaria (written communication from Professor V. A. McKusick, October 1972).

In the family reported on by Black,[14] FCU had a late onset. All our patients were questioned for evidence of deafness or renal disease, with negative results.

No definite conclusions can be drawn about the pathogenesis of FCU. Circumstantial evidence suggests that humoral factors play an important role in eliciting symptoms.

First, the syndrome is not a purely urticarial disorder, but appears to be a systemic reaction with fever, chills and joint symptoms.

Second, leukocytosis due to a rise in polymorphonuclear leukocytes seems to be a constant accompaniment of the generalized reaction.

Third, the reaction can be prevented by prior administration of *Pseudomonas* polysaccharide complex,[5] suggesting the involvement of complement factors.

Fourth, cold exposure leads to a decrease in the chymotrypsin-inhibiting capacity, either due to the generation of chymotrypsin-like enzymes in plasma or the release of intracellular (lysosomal) proteases. An increase in trypsin-inhibiting capacity was also observed as a result of cold exposure. Increased levels of trypsin inhibitors are found in patients with severe infections and after chest surgery.[17]

These changes suggest a certain similarity with hereditary angioneurotic edema and hereditary pulmonary emphysema, in which an enzyme inhibitor is congenitally absent.

In FCU this is not the case, but it can be postulated that patients with the disease have an easily activated protease or factors in their fibrinolysis, kallikrein or complement systems that activate them. Therefore, a study of the role of proteases and active components in the clotting-fibrinolysis, kallikrein and complement systems in FCU is now under way and the results will be published.

It is also hoped that the study of linkage of the cold urticaria locus, with a number of genetic markers, which has produced interesting but inconclusive findings,[10] (chapter 5) can be extended to other families with the same disease.

SUMMARY

Eleven patients with familial cold urticaria were found among 19 subjects in three generations of a Dutch family. There was autosomal dominant inheritance. The symptoms, which started during the first years of life, were noticed one half to three hours after generalized exposure to cold air.

The patients had erythematous plaques, urticarial lesions, petechiae, and edematous swellings of the exposed parts, often accompanied by fever, chills and joint complaints. Symptoms ranged from slight to incapacitating.

In two patients, the symptoms could be reproduced in the cold room. A leukocytosis preceded and accompanied the symptoms. During cold exposure, there was a sharp decrease in the level of chymotrypsin-inhibiting capacity in the plasma, which suggests that during the active phase of the disease a chymotrypsin-like enzyme is released.

REFERENCES

1. Kile RL, Rusk HA: A case of cold urticaria with an unusual family history. *JAMA* 114: 1067–1068, 1940.
2. Urbach A, Hermann MF, Gottlieb PM: Cold allergy and cold pathergy. *Arch Dermatol Syphilol* 43: 366–374, 1941.
3. Witherspoon FG, White CB, Bazemore JM: Familial urticaria due to cold. *Arch Dermatol Syphilol* 58: 52–55, 1948.
4. Rodin HH: Sensitivity to cold. *Arch Dermatol Syphilol* 63: 152–155, 1951.
5. Tindall JP, Beeker SK, Rosse WF: Familial cold urticaria: A generalized reaction involving leucocytosis. *Arch Intern Med* 124: 129–134, 1969.
6. Derbes VJ, Coleman WP: Familial cold urticaria. *Ann Allergy* 30: 335–341, 1972.
7. Castelain PY: Urticaire familiale au froid. *Bull Soc Fr Derm Syph* 78: 525–526, 1971.
8. Juhlin L, Shelley WB: Role of mast cell and basophil in cold urticaria with associated systemic reactions. *JAMA* 177:371–377, 1961.
9. Houser DD, et al: Cold urticaria: Immunologic studies. *Am J Med* 49: 23–33, 1970
10. Doeglas HMG, et al: Familial cold urticaria: Linkage analysis. *J Med Genet* 11, 31–34, 1974.
11. Doeglas HMG: Familial cold urticaria. *Arch Dermatol* 107: 136, 1973.
12. Donaldson VH: Serum inhibitor of C'1 esterase in health and disease. *J Lab Clin Med* 68: 369–382, 1966.
13. Colman RW, Mason JW, Sherry S: The kallikreinogen-kallikrein enzyme stystem of human plasma. *Ann Intern Med* 71: 763–773, 1969.
14. Black JT: Amyloidosis, deafness, urticaria and limb pains: a hereditary syndrome. *Ann Intern Med* 70: 989–994, 1969.
15. McKusick VA, Goodman RM: Pinnal calcification: Observations in systemic diseases not associated with disordered calcium metabolism. *JAMA* 179: 230–232: 1962.
16. Shepard MK: *Cold Hypersensitivity.* Birth defects original articles series VII, 8. Baltimore, The National Foundation-March of Dimes, 1971, vol 8, p 352.
17. Talamo RC: The α_1-antitrypsin in man. *J Allergy* 48: 240–250, 1971.

CHAPTER 5

A KINDRED WITH FAMILIAL COLD URTICARIA. LINKAGE ANALYSIS *

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INTRODUCTION

Familial cold urticaria (FCU) is a rare autosomal dominant disease of which six pedigrees have been published from the USA.[1-6] In addition, a brief case report from France [7] mentioning dominant transmission through three generations is available.

Onset is shortly after birth. The symptoms occur on generalized exposure to cold air after an interval of one half to several hours. The skin lesions begin on the exposed skin and consist of erythematous macules and plaques, urticarial lesions and sometimes petechiae. After severe or prolonged exposure the skin lesions are accompanied by chills, fever, cushion-like edema of the extremities and joint complaints. The symptoms may last for up to 48 hours and are incapacitating in some cases. The only abnormal laboratory finding is a leucocytosis which precedes and accompanies the cold reaction. The symptoms tend to diminish with advancing age.

The disease is easily differentiated from acquired contact cold urticaria which is less rare, occurs sporadically and is characterized by symptoms like those seen during histamine release.

Recently the clinical findings in a kindred with FCU have been reported from the Netherlands.[8,9] In this family linkage studies were performed and these results are presented here. This is the first family with FCU in which linkage studies have been done.

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MATERIAL AND METHODS

Fig. 1 shows part of a larger pedigree. The complete pedigree covers more than 150 years and contains 108 descendants of a couple who were born in 1824. The family lives in the north-eastern part of the Netherlands in a rural area between the city of Groningen and the German border. Some members have moved to a nearby province. The family data were obtained from local registry offices, which in the Netherlands contain data from 1810 onwards. The complete kindred has been presented in another publication [9] (see chapter 4) but only one branch (XC) of the family is of relevance to the present discussion, as it includes living affected members. This point was established by investigation of the entire kindred mostly by home visits, but relying on mail and telephone contact in a few cases.

At each contact the family history was cross checked with special attention to details about deceased members. Of all members of branch XC, blood was drawn for linkage studies (5 ml clotted blood, 5 ml in EDTA solution, and 5 ml in acid citrate dextran solution). The tubes were transferred to laboratories in Amsterdam and Leiden within 12 hours. The techniques used for the determination of the different markers are as described in the literature.[10,11]

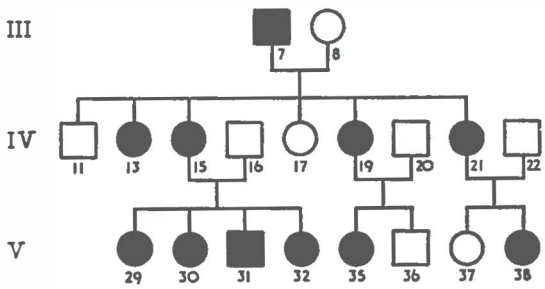

Fig. 1

BRANCH XC (Fig. 1)

All affected living members occur in branch XC of the larger kindred of Doeglas [9] *et al.* of which the senior member is still alive (III.7). His older sister had severe symptoms during life, but her only son and his offspring are free of the disease.

The father of III.7 was affected and was apparently the first case, thus possibly representing a new mutation although this cannot be regarded

as established because of the circumstantial nature of evidence collected about generations too remote in time. Apart from III.7 and his affected sister, there was one other sib who survived infancy, but did not show the disease.

Fig. 1 shows 11 cases of familial cold urticaria ranging from slight to severely incapacitating, among 19 individuals. Their case histories have been described extensively elsewhere [9] (chapter 4). There is autosomal dominant inheritance of the trait in this family.

LINKAGE ANALYSIS

All the members of branch XC were investigated for the following 31 genetic markers in order to perform linkage analysis between each of the loci or chromosomal regions for these markers and the cold urticaria (CU) locus: ABO, MNS, P, Lu, Rh, K, Fy (blood groups); Gc, Gm, Inv, Hp, Tf, C'3, E_1, E_2 (serum groups); AcP, ADA, AK, PGD, PGM_1, GPT (red cell enzymes); and polymorphic chromosomes 1, 3, 4, 9, 13, 14, 15, 16, 21, and 22. The phenotypic distribution of the variable markers in this pedigree are shown in Table 1. Lod scores calculated [12,13] for the linkage of the cold urticaria locus with these different marker systems are presented in Table 2.

The data suggest that the loci for Inv, Hp, C_3', AcP, MNS, and Fy are probably not within measurable distance from the CU locus. More information is needed to define the linkage relationships between the CU locus and the loci for Rh and ABO, respectively. Based on the present material a linkage between the CU locus and the loci for red cell glutamic pyruvic transaminase (GPT) and Gc serum group respectively cannot be excluded. Polymorphisms of chromosomes 3, 13, and 22 were informative for linkage and it appears that a close linkage can be excluded between each of their C-banding and/or satellite regions and the CU locus.

All the basic data collected during the present investigations are included in this paper, in order to facilitate a comparative and combined treatment with similar data to be collected in future from other families.

TABLE 1. PHENOTYPE

	sex	CU	serum types					red cell enzymes			
			Gm[b]	Inv[c]	Gc	Hp	C'3	E_1[d]	AcP	AK	GPT	PGM_1
III.7	M	+	aa	++	1–1	2–1	S	U	BA	1	1	1
III.8	F	−	aa	−−	2–1	2–1	FS	U	B	1	2–1	1
IV.11	M	−	aa	++	1–1	2–1	S	U	B	1	1	1
IV.13	F	+	aa		1–1	2–2	S	U	B	1	2–1	1
IV.15	F	+	aa	++	1–1	2–1	FS	U	BA	1	1	1
IV.16[h]	M	−	aa	−−	2–2	1–1	S	U	B	1	2–1	2
IV.17	F	−	aa	−−	1–1	1–1	S	U	B	1	2–1	1
IV.19	F	+	aa	++	2–1	2–1	S	U	BA	1	1	1
IV.20[h]	M	−	ab	++	2–1	2–2	S	U	BA	1	2–1	2–1
IV.21	F	+	aa	++	1–1	2–2	S	U	BA	1	2–1	1
IV.22[h]	M	−	aa	++	1–1	2–2	FS	I	BA	2–1	1	1
V.29	F	+	aa	−−	2–1	1–1	FS	U	B	1	1	2–1
V.30	F	+	aa	++	2–1	2–1	S	U	B	1	1	2–1
V.31	M	+	aa	++	2–1	1–1	FS	U	B	1	1	2–1
V.32	F	+	aa	++	2–1	2–1	S	U	B	1	1	2–1
V.35	F	+	ab	++	1–1	2–1	S	U	BA	1	1	1
V.36	M	−	aa	++	2–1	2–1	S	U	A	1	1	1
V.37	F	−	aa	++	1–1	2–2	FS	I	BA	1	2–1	1
V.38	F	+	aa	−−	1–1	2–2	FS	U	BA	2–1	1	1

[a] Tf, E_2, ADA, PGD, P, Lu, K, and chromosomes No. 1, 3, 4, 9, 15, 16 were invariable in this pedigree.

[b] aa = Gm(a−x−f+n+g−b+); ab = Gm(a+x+f+n+g+b+).

[c] ++ = Inv(1+a+); −− = Inv(1−a−).

[d] E_1 = pseudocholinesterase$_1$: U = usual; I = intermediate.

[e] C = C-banding: presence (+) or absence (−) of C-band in one or the other or both of the No. 3 chromosomes.

[f] A = chromosome No. 13 without a detectable marker on it; C is No. 13 with fluorescent short arms; S is No. 13 with a fluorescent satellite.

[g] S = Satellite: presence (+) or absence (−) of a fluorescent satellite on the corresponding chromosome.

č = variant of c.

[h] Related by marriage only.

DISTRIBUTION IN BRANCH XC[a]

red cell antigens				marker chromosomes				
ABO	MNS	Rh	Fy	No. 3[e] C	No. 13[f] A–C–S	No. 14[g] S	No. 21[g] S	No. 22[g] S
O	NNS−	CcDee	a+	+/+	SA	−/−	−/−	+/−
A_1	MMS+	CcDEe	a+	−/−	CA	−/−	−/−	−/−
O	MNS+	CcDee	a+	+/−	AA	−/−	−/−	+/−
A_1	MNS−	CcDee	a+	+/−	SA	−/−	−/−	+/−
A_1	MNS+	CcDEe	a+	+/−	CA	−/−	−/−	−/−
O	NNS−	Cčdee	a+	+/+	CA	−/−	−/−	+/−
A_1	MNS+	ccDEe	a+	+/−	CS	−/−	−/−	+/−
O	MNS−	CcDee	a+	+/−	AA	−/−	−/−	+/−
O	MMS+	ccdee	a−	+/−	AA	+/−	+/−	−/−
O	MNS+	CCDee	a+	+/−	CS	−/−	−/−	−/−
O	MMS+	ccDEe	a+	+/−	CA	−/−	−/−	+/−
O	NNS−	CCDee	a+	+/−	CA	−/−	−/−	+/−
A_1	NNS−	CCDee	a+	+/−	CC	−/−	−/−	−/−
O	MNS+	CcDee	a+	+/−	CA	−/−	−/−	+/−
O	MNS+	CcDEe	a−	+/+	CA	−/−	−/−	−/−
O	MNS+	ccdee	a+	+/−	AA	+/−	+/−	−/−
O	MNS+	CcDee	a+	+/+	AA	+/−	+/−	+/−
O	MNS+	CcDEe	a−	+/−	CS	−/−	−/−	+/−
O	MNS+	CcDee	a+	+/+	SA	−/−	−/−	−/−

TABLE 2. LOD SCORES FOR COLD URTICARIA LOCUS AND MARKER SYSTEMS (COMPOSITE)

marker System	No. of children scored	recombination fraction					
		0.00	0.05	0.10	0.20	0.30	0.40
Inv	11	−∞	−1.517	−0.758	−0.165	−0.023	+0.043
Hp	9	−∞	−1.630	−0.866	−0.264	−0.057	−0.004
C'3	4	−∞	−1.442	−0.887	−0.388	−0.152	−0.036
Gc	1	+0.301	+0.279	+0.255	+0.204	+0.146	+0.079
AcP	11	−∞	−4.907	−3.219	−1.667	−0.888	−0.409
GPT	2	+0.602	+0.558	+0.511	+0.408	+0.292	+0.158
ABO	4	−∞	−0.163	+0.066	+0.214	+0.216	+0.140
MNS	8	−∞	−2.884	−1.774	−0.776	−0.304	−0.072
Rh	12	−∞	−0.790	−0.096	+0.368	+0.421	+0.280
Fy	6	−0.297	−0.178	−0.110	−0.044	−0.014	−0.003
chromosome No. 3	6	−∞	−3.442	−2.285	−1.184	−0.596	−0.230
No. 13	9	−∞	−3.885	−2.474	−1.174	−0.525	−0.168
No. 22	8	−∞	−3.185	−2.071	−1.050	−0.531	−0.212

SUMMARY

Familial cold urticaria is an autosomal dominant hereditary disease of which seven pedigrees have been published, six from the USA and the present one from Holland. Onset is shortly after birth. The patients develop skin symptoms sometimes accompanied by fever, chills, joint symptoms and edema, approximately one half to three hours after generalized exposure to cold.

In this paper the linkage data from the Dutch family are described; three generations containing 11 affected persons were studied in relation to 31 markers. While the data are inconclusive, they are presented here from the point of view of eventual accumulation when other families are studied in the same way.

REFERENCES

1. Kile RL, Rusk HA: A case of cold urticaria with an unusual family history. *JAMA* 114, 1067–1068, 1940.
2. Urbach E, Hermann MF, Gottlieb PM: Cold urticaria and cold pathergy. *Arch Dermatol Syphilol* 43, 336–374, 1941.
3. Witherspoon FG, White CB, Bazemore JM et al: Familial urticaria due to cold. *Arch Dermatol Syphilol.* 58, 52–55, 1948.
4. Rodin HH: Sensitivity to cold. *Arch Dermatol Syphilol* 63, 152–155, 1951.
5. Tindall JP, Beeker SK, Rosse WF: Familial cold urticaria. A generalized reaction involving leukocytosis. *Arch Intern Med* 124, 129–134, 1969.
6. Derbes VJ, Coleman WP: Familial cold urticaria. *Ann Allergy* 30, 335–341, 1972
7. Castelain PY: Urticaire familiale au froid. *Bull Soc Fr Dermatol Syphiligr* 78, 525–526, 1971.
8. Doeglas HMG: Familial cold urticaria. *Arch Dermatol* 107, 136–137, 1973.
9. Doeglas HMG, Bleumink E: Familial cold urticaria. Clinical findings. *Arch Dermatol* 110, 382–388, 1974.
10. Giblett ER: *Genetic markers in human blood.* Oxford, Blackwell Scientific Publications, 1969.
11. Pearson PL, Gerards JPM, Linde AGJM van der: *Human chromosome polymorphism*, in: Symposia Medica Hoechst, Modern Aspects of Cytogenetics: Constitutive Heterochromatin in Man, New York, Schattauer, 1973, pp 201–213.
12. Morton NE: Sequential tests for the detection of linkage. *Am J Hum Genet* 7, 277–318, 1955.
13. Maynard-Smith S, Penrose LS, Smith CAB: *Mathematical tables for research workers in human genetics.* London, Churchill, 1961.

SUMMARY

This study describes a combined clinical, laboratory and experimental approach of the problems of 141 patients with chronic urticaria, collected over a three-year period in a Dermatology department.

In the first chapter the classification, clinical methods of study and the incidence are discussed. In 55 % of the patients physical urticarias could be demonstrated. Atopy was not more frequent than in a control group, except for a statistically significant increase in patients with idiopathic urticaria and urticaria factitia. Drugs were not a frequent cause of chronic urticaria. Only in one case of penicillin allergy, a direct relationship between drug use and chronic urticaria could be demonstrated. The possibility had to be considered that the removal of dental periapical granulomas and the treatment of oxyuriasis, was responsible for the 'prompt and permanent' cure of some patients with chronic urticaria. In two patients chronic urticaria appeared to be the introductory phenomenon of systemic lupus erythematosus.

In a second section the role of aspirin in the pathogenesis of chronic urticaria is examined. Provocation tests with aspirin were positive in 26 % of 131 patients. The incidence of aspirin sensitivity was higher in cholinergic urticaria (52 %) and delayed pressure urticaria (43 %) than in other groups. In patients sensitive to aspirin similar reactions were seen after provocation tests with the azo dye tartrazine, benzoates and the analgesics indomethacin and paracetamol. Sodium and phenyl salicylate gave reactions in some aspirin sensitive patients as well. There was no correlation between aspirin sensitivity and nasal polyposis, sinusitis, asthma or atopy in this group of patients.

A diet free of tartrazine, benzoates and salicylates cured the urticaria in about half of the aspirin sensitive patients. Aspirin has to be considered a secondary exacerbating factor in chronic urticaria.

The third chapter gives the results of protease inhibitor determinations in the plasma of patients with chronic urticaria and controls. Patients with acquired cold urticaria had significantly decreased levels of α_1-antitrypsin and total antitrypsin activity. In patients with acquired angioneurotic edema, α_1-antitrypsin levels and antichymotrypsin activities were lowered with less significant decreases in antitrypsin and antikallikrein activities. Levels of the complement factors C1 esterase, C3 and C4 and

α_2-macroglobulin were normal in all groups. There was no correlation between increased skin sensitivity to kallikrein and deficiencies of protease inhibitors.

In chapter four the clinical and genealogical study of a family with familial cold urticaria living in the Netherlands is described. There were 11 patients among 19 family members in three generations. The cold urticaria trait was inherited as an autosomal dominant. The symptoms were reproduced in two patients by exposure to a cold room at 6° C for several hours. They showed a sharp decrease in chymotrypsin inhibiting capacity during cold exposure.

The final section describes a linkage analysis of this family. All 19 members were studied in relation to 31 genetic markers, consisting of blood groups, serum groups, red cell enzymes and polymorphic chromosomes. The data were inconclusive, but are represented from the point of view of eventual accumulation when other families are studied in the same way.